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PROCEEDINGS

of the

ASSOCIATION FOR RESEARCH IN OPHTHALMOLOGY, INC.

Section Meetings

1957-1958

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FACTORS INFLUENCING THE MOVEMENT OF FLUORESCEIN IN THE CORNEA*

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Toronto, Ontario

Fluorescein has been used by many experimenters to assess the permeability of the cornea. Bellarminoff's[1] work, in 1893, set a pattern for a number of workers. He studied the penetration of fluorescein from without inward under varying conditions, which included removal of the epithelium and raising the intraocular pressure. He concluded that the cornea was permeable from without inward, that removal of corneal epithelium increased the permeability, and that raising the intraocular pressure lowered the permeability.

Since that time many other workers have studied corneal permeability with dyes. Nakamura,[2] in 1923, investigated the effect of the tonicity of the dye solutions. His broad conclusions, since confirmed by other workers, were that solutions of tonicity less than 0.6-percent NaCl or greater than 1.3-percent NaCl penetrated more rapidly. This he considered to be due to damage of the corneal epithelium and endothelium.

Fischer,[3] 1928-1931, after a long series of experiments which included dye studies, postulated unidirectional permeability of the cornea from without inward. At almost the same time Ridley's[4] experiments with fluorescein suggested that the endothelium was permeable. These experiments have ben criticized on the grounds that the endothelium may have been damaged. But, in 1947, Ricca[5] also demonstrated permeability from within outward and reiterated that the endothelium was permeable.

Cogan and Hirsch[6] studied corneal permeability using aniline, a dye of considerably larger particle size than fluorescein. They found that penetration depended on the state of dissociation. Their experiments are regarded as evidence supporting the phase solubility theory of corneal permeability.

Maurice,[7] in 1953, reviewed the literature on corneal permeability and made a number of constructive criticisms of dye experiments. He suggested that a quantitative approach is necessary in permeability studies and that account must be taken of the tonicity of the solutions used.

In our experiments we have tried to modify the techniques of earlier workers and have attempted a quantitative evaluation of the roles of the various layers of the cornea. Our method has measured penetration time rather than assessment of aqueous concentration of the dye. Our results are therefore recorded in time, representing speed of movement of the dye.

Method

All experiments were done on rabbits, anesthetized with intravenous Nembutal. To measure anterior to posterior movement sodium fluorescein in 20-percent concentration was applied on the end of an applicator stick to the surface of the cornea for 30 seconds. The eye was observed by slitlamp microscope. The time from the start of the application until the dye was first seen as a stream flowing down the posterior surface of the cornea was taken (fig. 1).

To measure posterior to anterior movement 0.05 cc. of 20-percent sodium fluorescein was injected in the anterior chamber. With the aid of the slitlamp microscope it was seen to advance through the cornea. The time from injection until the anterior face of the advancing fluorescein struck the second bright surface in the slitlamp beam was taken (fig. 2).

When the effect of Pontocaine on the epithelium was tested, this drug was applied

* From the Department of Ophthalmology, University of Toronto.

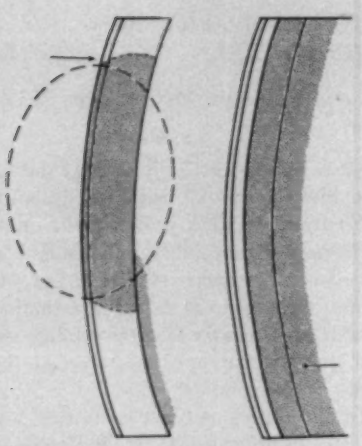

FIG. 1 FIG. 2

Fig. 1 (Beswick, Langley, and McCulloch). The dye has been applied to the anterior surface of cornea. It has penetrated the cornea and is flowing downward in the aqueous.

Fig. 2 (Beswick, Langley, and McCulloch). Movement of dye anteriorly through the cornea after injection in the anterior chamber.

to the surface of the cornea in 0.5-percent solution two minutes before the fluorescein was applied to the epithelium or injected in the anterior chamber. To test the action of Pontocaine on the posterior surface it was injected in the anterior chamber with the fluorescein or one minute before the dye was applied to the epithelium.

To control the ocular pressure a No. 26 gauge needle, with a hole in the side of its shaft, was inserted in the anterior chamber. The needle was driven across the chamber, through the cornea opposite to the point of entry, and into a rubber tube. Fluid could then be injected into the anterior chamber from one end of the needle, while from the other end a system with a reservoir controlled the ocular pressure (fig. 3).

To remove the epithelium it was first marked with a trephine and then scraped off with a Tooke knife. When the endothelium was broken this was done by drawing the needle across it. A standard endothelial lesion is difficult to produce. In our experience a small lesion gives the most clearcut result.

If the endothelium had been abraded at one point the fluorescein moved forward, through the cornea, more quickly from that point than from elsewhere on the endothelial surface. An advancing "mound" of fluorescein was produced, the peak touching the epithelial surface ahead of the face of fluorescein from intact endothelium (fig. 4). Just after the peak touched the anterior corneal light reflex fluorescein appeared on the surface of the cornea and tended to run downward as a film of dye on the surface.

The effect of removing the epithelium on the posterior to anterior movement of dye can be seen while the dye is advancing through the cornea. Behind intact epithelium the advancing face of dye moves ahead of the dye behind removed epithelium. As a result the face of the dye appears as a cup-like cavity behind the area of bared Bowman's membrane (fig. 5). The time studies represent readings taken under intact and removed epithelium.

The series shown in Table 1 were done.

Certain of these times were compared. Table 2 shows the significance of the differences.

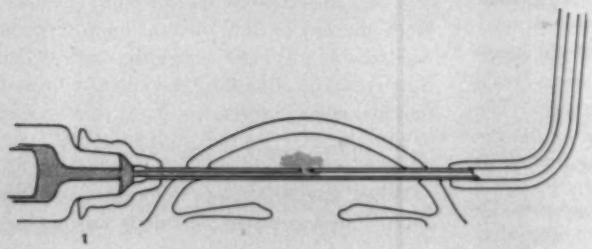

Fig. 3 (Beswick, Langley, and McCulloch). System to control intraocular pressure after injecting dye in the anterior chamber.

FIG. 4

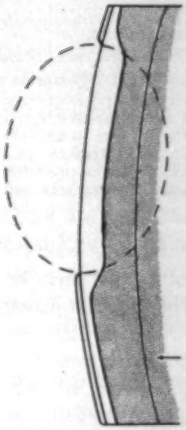

FIG. 5

Fig. 4 (Beswick, Langley, and McCulloch). The more rapid advance of dye from the anterior chamber through the cornea in front of an abrasion of the endothelium.

Fig. 5 (Beswick, Langley, and McCulloch). Slower advance of dye through the stroma behind an area of removed epithelium.

The points which should be noted in the tables are:

a. When the epithelium was removed, the time of movement of fluorescein from outside inward was decreased; on the other hand, the time of movement of fluorescein from inside out was increased.

b. When the intraocular pressure was raised by 33 mm. Hg, the time of movement of fluorescein from without inward increased but the time of movement of fluorescein from within outward decreased.

c. When the endothelium was abraded, the time of movement of fluorescein from within outward decreased. We were not able to establish an experimental procedure for testing the movement of fluorescein in the other direction, after scratching the endothelium.

d. The application of Pontocaine to the epithelium decreased the time of movement of fluorescein from the outside in and from inside out. However, on reviewing the experimental procedures we would feel that the effects we were getting were in part mixed, that is, on both the epithelium and endothelium. When studying the epithelial effect the Pontocaine was dropped for two minutes on the surface before the fluorescein was applied. If the Pontocaine moved

TABLE 1

RESULTS

Series							
Anterior to Posterior				Posterior to Anterior			
a. Control b. Pontocaine to epithelium c. Pontocaine to endothelium d. Epithelium scraped off e. Low intraocular pressure (20 mm. Hg) f. High intraocular pressure (53 mm. Hg)				a. Control (20 mm. Hg) b. Pontocaine to epithelium c. Pontocaine to endothelium d. Epithelium scraped off e. High ocular pressure (53 mm. Hg) f. Endothelium scraped			
Times							
Anterior to Posterior				Posterior to Anterior			
	N	M(sec.)	s		N	M(sec.)	s
Control	10	309	87	Control	10	210	5.2
Pontocaine to epithelium		227	36	Pontocaine to epithelium		171	16
Pontocaine to endothelium		303	17	Pontocaine to endothelium		192	26
Epithelium scraped off		226	36	Epithelium scraped off:			
Low pressure		298	31	Side		259	16
High pressure		348	22	Center		462	37
				High Pressure		147	12
				Endothelium scraped:			
				Intact		316	42
				Affected		165	19

TABLE 2

SIGNIFICANCE OF THE DIFFERENCE

Anterior to Posterior		Posterior to Anterior	
Pontocaine to epithelium vs. control	2.6	Pontocaine to epithelium vs. control	7
Epithelium removed vs. control	2.6	Pontocaine to endothelium vs. control	2
Pontocaine to endothelium vs. control	0.4	Intact epithelium vs. epithelium off	16
Low vs. high pressure	4	High vs. low pressure	15
		Endothelium scraped vs. intact	10

at anything like the speed that fluorescein moves it would have reached the endothelium during the course of the experiment, resulting in a mixed epithelial-endothelial effect. When Pontocaine was applied to the endothelium it was injected a minute before fluorescein was applied to the cornea or injected with the fluorescein in the anterior chamber. Again both surfaces of the cornea would be affected during the experiment and no conclusion as to the effect on either surface, separately, could be drawn.

e. Besides Pontocaine two poisons, iodoacetic acid and cyanide, were tested. It was felt that, for the same reasons, the results could not be interpreted.

Discussion

It has been suggested that the stroma is permeable to water and salt.[8] Major barriers to permeability through the cornea are the epithelium and endothelium. Three factors limiting permeability through the epithelium and endothelium can be considered. These surfaces may allow particles to pass by virtue of pores; the pore size at each surface will then be the factor determining permeability. The second limiting quality may be related to phase solubility.[9] The fat and water solubility of a test substance, and the ease of change of phase of the test substance, may be a factor in the speed of penetration. Finally, the maintenance of an electric potential between epithelium and stroma and between stroma and endothelium may control the movement of electrolytes. A negative ion would show movement toward a positive and away from a negative charge. In the light of these three possible controlling factors, where do the results of the present experiment lie?

The fact that damage to the epithelium, or removing it, increased the speed of anterior to posterior penetration of the dye is compatible with all three factors. The pores would be opened, new ones made. With regard to phase solubility, fluorescein may be considered a water-soluble dye, impeded by the epithelium. Damage or destruction of this layer would then speed penetration. Finally, as regards electric charge, a positive charge in the epithelium and a negative charge in the stroma would slow entrance of the dye. Damage or loss of epithelium would destroy the electric differential and speed the anterior to posterior movement of the dye.

The same discussion related to all three factors would apply to penetration of the endothelium in a posterior to anterior direction.

The fact that loss of the epithelium slowed the posterior anterior movement of the dye in the stroma is worthy of special consideration. The dye had not reached the epithelium before the effect was apparent. Changes in the pores or in phase solubility could not account for this. If an electrical effect was being demonstrated the removal of epithelium must have changed the potential in the neighboring stroma, slowing the advance of the dye.

The fact that raising the ocular pressure speeded the posterior anterior movement of the dye, but slowed the anterior posterior movement, is difficult to explain by any of the three factors. Changes in the pores or variation in phase solubility would hardly

account for faster movement in one direction, but slower movement in the other. It is hard to conceive of electric charges causing this increased speed outward, but decreased speed inward.

The easiest way to explain the findings in these experiments is by assuming that the epithelium and endothelium are maintaining a flow of water, each toward its neighboring corneal surface.[10] At the epithelial surface this may be substantial, at the endothelial surface this may be largely balanced by the ocular pressure tending to maintain a posterior to anterior flow of water across the endothelium. Florescein can then be considered as diffusing in water which is itself moving. (A dye placed in a river spreads out, but advances faster downstream than upstream.) Damage or removal of the epithelium stops this flow of water and speeds the movement of dye inward, but slows its movement outward. The same situation occurs at the endothelium. Here, if the surface is damaged, the intraocular pressure forces fluid and dye through the damaged area into the stroma. Also, raising ocular pressure adds to the forces moving water anteriorly through the endothelium. At the posterior surface there is, then, slower movement of dye posteriorly or faster movement anteriorly as a result of raising ocular pressure.

Considering the cornea as 0.5 mm. thick, the speeds of movement of the dye can be given in mm. per second.

Anterior to posterior 0.00162
Posterior to anterior 0.00238

If the epithelium is removed the changes in rate of movement of the dye are:

Anterior to posterior	0.00162	
	0.00221	0.00059
Posterior to anterior	0.00193	
	0.00108	0.00085
Average =		0.00072 mm. per sec.

If the endothelium is removed the change is:

Posterior to anterior 0.00158
0.00303 0.0015

The epithelium produces a flow of 0.00072 mm. per second in a posterior to anterior direction, the endothelium a flow of 0.0015 mm. per second in an anterior to posterior direction.

If the intraocular pressure is raised 33 mm. Hg the rate of movement in the anterior to posterior direction is slowed from 0.00168 to 0.00144 = 0.00024 mm. per second. In the posterior to anterior direction it is speeded from 0.00108 to 0.00193 = 0.00085 mm. per second. Averaging these, there is a flow produced of 0.00055 m/sec. in a posterior to anterior direction, representing a rate of 0.000017 mm. per sec. per mm. Hg intraocular pressure.

Conclusion

It is suggested that the epithelium is maintaining a substantial flow of water outward, and the endothelium a flow inward which is partially balanced by an outward flow produced by the intraocular pressure.

Summary

1. The rate of corneal penetration of fluorescein has been studied in rabbits under varying conditions.

2. The effects produced by the corneal epithelium, endothelium, and intraocular pressure have been considered.

3. It is suggested that the cornea epithelium maintains an outward flow of water and the endothelium an inward flow, the latter practically balanced by an outward flow produced by the intraocular pressure.

The Medical Arts Building.

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THE RETINAL CIRCULATION TIME*

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AND

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Adler has suggested that blood flow studies through the eye would be invaluable.[1] He has pointed out the similarity of conditions in the eye with those in the skull. The Monroe-Kellie doctrine of an invariability of the volume of the intracranial contents is based on the firm rigidity of the skull.[1] The total intracranial volume can vary little; and so, in order to have a greater volume of blood to flow through the brain, the velocity of blood flow must be increased. The sclera has some elasticity; and to this extent the pressure relationships in the eye differ from the Monroe-Kellie doctrine.

In other organs such as the kidney, liver, or muscle, where the whole organ can expand, the vessels can dilate readily if the demand for oxygen is increased. In the eye, the situation is probably somewhere in between that found in the skull and that in other organs; if there is an increased demand for oxygen, it can be met by an increase in the blood volume and by acceleration of blood flow through the eye.

Circulation times in various parts of the body have been studied well with a variety of techniques. Most of us are familiar with arm to tongue circulation time using saccharin or Decholin, and with arm to lung circulation time using ether or paraldehyde. The circulation times of various organs have been determined; for example, the average coronary circulation time is eight seconds[2] and the average cerebral circulation time is three seconds.[1] But, according to Adler, no one has previously measured the retinal circulation time.[1]

We have devised a technique whereby we can measure the circulation time of the cat retina. One percent aqueous trypan blue is injected rapidly into one internal carotid artery of an anesthetized cat with pupils

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* From the Division of Ophthalmology, Department of Surgery, Stanford University Medical School. Presented in part before the Association for Research in Ophthalmology, Western Section, on November 14, 1957, at San Francisco, California.

dilated by mydriatic drugs. At the same time, a second person with stop watch in hand is carefully focussing with an ophthalmoscope on a branch of the central retinal artery and a branch of the central retinal vein as they cross the disc of the eye on the same side as the injected artery. At the moment the artery turns deep blue at the disc margin, the stop watch is pressed. From 1.9 to 2.1 seconds later, the vein at the disc margin also turns deep blue and the watch is stopped (Figs. 1, 2, and 3). The trypan blue takes approximately two seconds to transverse the capillary bed of the retina and return to the disc margin.

More than 100 trials have been made on 17 cats. To ascertain whether the difference of viscosity of the solution from that of blood was an important factor, blood from the cat was heparinized and mixed with trypan blue to make a two-percent solution. The color of the blood-trypan blue solution was not quite as dark as the one percent saline-trypan blue solution. The circulation time was the same as before, but the end points did not seem as sharp and as easily read as with the aqueous solution. The saline-trypan blue solution was therefore much more satisfactory and was used for all remaining tests.

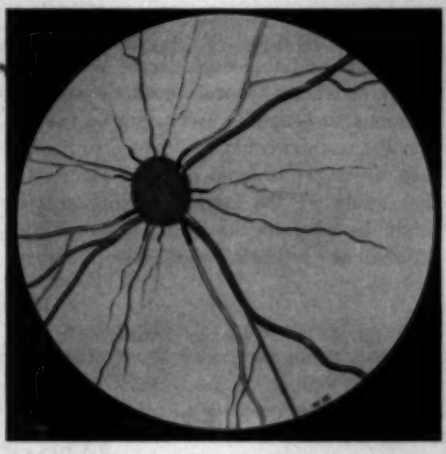

Fig. 2 (Chao and Flocks). The trypan blue has entered the arterioles. The stop watch is started at this point.

Increasing the intraocular pressure by compression of the sclera slows the circulation time, and lowering the intraocular pressure by paracentesis apparently makes the circulation time faster.

Our study of the circulation time of the cat retina is continuing. We are not yet sure

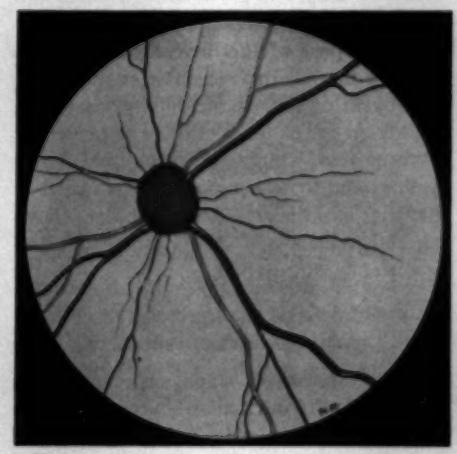

Fig. 1 (Chao and Flocks). Diagram of cat fundus prior to injection of trypan blue. The veins appear slightly darker than the arterioles.

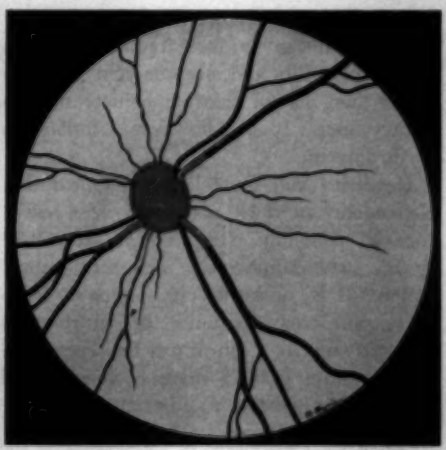

Fig. 3 (Chao and Flocks). The trypan blue is returning in the veins and has almost reached the disc. The stop watch is stopped at this point.

whether the method is sensitive enough to determine changes due to the effects of parenterally administered drugs. We do not yet know whether more innocuous indicator solutions such as fluorescein can be used so that the method could be adapted to the human retina? If it could be, it is conceivable that study of the retinal circulation time might be of value in such pathologic conditions as retinitis pigmentosa, diabetic retinopathy, and macular degeneration.

Summary

A technique is described for determining the circulation time of the retina. The circulation time of the cat retina is approximately two seconds.

2018 Webster Street.
850 Middlefield Road.

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OLEOANDOMYCIN*

J. Dumas, M.D., I. L. Fielding, and H. L. Ormsby, M.D.
Toronto, Ontario

Oleoandomycin phosphate is a crystalline substance obtained from a strain of streptomyces antibioticus. It is water soluble up to five percent, and stable from a pH of 1.1 to 9.0. Thus the drug is not inactivated in the stomach or duodenum.[1] Oleoandomycin is reported[2] to inhibit most gram-positive bacteria, but has only a slight inhibiting effect on gram-negative bacteria, rickettsiae, and larger viruses. The spectrum of activity on micro-organisms is therefore wider than that of penicillin and streptomycin, but narrower than that of chloramphenicol and the tetracyclines. It is, however, similar to erythromycin.

Needham and Geraci[3] have shown that oleoandomycin does not have cross-resistance to penicillin, streptomycin, oxytetracycline, chloramphenicol, chlortetracycline, polymixin B, or bacitracin. There is, however, some cross-resistance to erythromycin and carbomycin. Clinically it has been used against those gram-positive bacteria found to be highly resistant to other antibiotics.

The absorption of oleoandomycin from the gastro-intestinal tract has been found to be very good, and blood concentrations of 0.4 to 4.0 μg./cc. have been reported after a single oral dose of 500 mg. Blood levels of 0.7 to 1.5 μg./cc. have been obtained after a single oral dose of 250 mg. In humans, no toxic manifestations have been seen, even when the drug was given in high doses and for prolonged periods.[2]

The purpose of this investigation was to compare the in vitro sensitivities with oleoandomycin of pathogenic staphylococci isolated from eye cultures to those of penicillin, erythromycin, chloramphenicol, and tetracycline; and to determine the penetration of the drug into the aqueous of rabbits following intravenous and subconjunctival injection, and by corneal bath.

In vitro sensitivities of pathogenic staphylococci

Fifty strains of staphylococcus aureus were isolated from ocular sources and were sub-cultured in Hartley's broth, incubated 24 hours, and diluted to 10^{-4}. The standard broth-dilution method of sensitivity testing

* From the Departments of Ophthalmology and Bacteriology, Faculty of Medicine, University of Toronto.

TABLE 1

SENSITIVITIES OF 50 STRAINS OF STAPHYLOCOCCUS AUREUS ISOLATED FROM EYE CULTURES

	Antibiotic concentrations in μg./cc.						
	*0.6	1.25	2.5	5.0	10	20	40
Chloramphenicol				88%	92%	92%	96%
Oleoandomycin	86%	86%	86%	90%	90%	92%	
Erythromycin	76%	80%	82%	82%	88%	90%	
Tetracycline	72%	78%	80%	80%	80%	80%	
Penicillin*	64%	78%	84%	86%	86%	88%	

* Units for penicillin.

was used. After 24 hours of incubation at 37°C., the pathogenic staphylococci were recorded as being either sensitive or resistant to a given strength of antibiotic. The dilutions chosen were based on the average blood serum levels and the range of bacterial sensitivities encountered in the laboratory. Four other antibiotics were compared with oleoandomycin.

RESULTS

It will be seen from Table 1 that 92 percent of strains of staphylococcus aureus were sensitive to 20 μg./cc., and 86 percent were sensitive to 0.6 μg./cc. Of the antibiotics under test, oleoandomycin was found to be more effective than erythromycin, tetracycline, and penicillin. The results for chloramphenicol were similar to those for oleoandomycin.

INTRAOCULAR PENETRATION OF OLEOANDOMYCIN

INTRAVENOUS INJECTIONS

In a group of 10 rabbits, a dose of 50 mg./kg. of oleoandomycin was injected intravenously. Blood and acqueous were removed at intervals to determine the concentration of the drug. A serial broth-dilution method of testing was used and Staphylococcus Peoria was the control organism.

It will be seen in Table 2 that after one, two, and four hours, the oleoandomycin concentration of the aqueous was about 50 percent of that of plasma. Six hours and 24 hours after injection, the plasma concentration was found to be lower than 0.30 μg./cc.

The intravenous injection was repeated with a dose of 100 mg./kg. in two rabbits. The aqueous concentration was also found to be 50 percent of that of plasma after one hour.

SUBCONJUNCTIVAL INJECTIONS

In another group of 12 rabbits (24 eyes) oleoandomycin was injected subconjunctivally. The aqueous concentration of oleoandomycin after subconjunctival dosage of 10 mg./cc. was high even after four hours (table 3). Severe local reaction was seen with this dosage, and edema followed by local necrosis appeared within five hours. Using a dosage of 2.5 mg./cc. subconjunctivally, therapeutic aqueous concentrations were obtained. Local reaction in the conjunctiva was still present with this reduced dosage.

TABLE 2

PLASMA AND AQUEOUS LEVELS AFTER INTRAVENOUS INJECTIONS OF OLEOANDOMYCIN (AVERAGE OF FOUR EYES)

Time after Injection (hr.)	Plasma Concentration μg./cc.	Aqueous Concentration μg./cc.
Dosage—50 mg./kg.		
1	4.80	2.40
2	2.40	1.80
4	1.20	0.90
6	<0.30	<0.30
24	<0.30	<0.30
Dosage—100 mg./kg.		
1	6.00	3.00

TABLE 3

AQUEOUS LEVELS OF OLEOANDOMYCIN FOLLOWING SUBCONJUNCTIVAL INJECTIONS (AVERAGE OF EIGHT EYES)

Time after Injection (hr.)	Average Aqueous Levels	
	Dose—10 mg./cc.	Dose—2.5 mg./cc.
	(μg/cc.)	(μg./cc.)
1	19.2	2.4
2	8.2	.1
4	1.2	.6

TABLE 4

AQUEOUS LEVELS OF OLEOANDOMYCIN FOLLOWING CORNEAL BATH (FIVE MINUTES) (AVERAGE OF FOUR EYES)

Dose—20 mg./cc.	
Time after Injection (hr.)	Average Aqueous Levels (μg./cc.)
½	7.2
1	4.8
2	2.2

CORNEAL BATH

A solution containing 20 mg./cc. of oleoandomycin phosphate was applied by corneal bath to 12 eyes for a period of five minutes. The aqueous was removed after intervals of one half, one, and two hours. Therapeutic concentrations were obtained in the aqueous, as shown in Table 4.

SUMMARY

1. Sensitivities of 50 strains of Staphylococcus aureus isolated from eye cultures were tested with chloramphenicol, oleoandomycin, erythromycin, tetracycline, and penicillin. Eighty-six percent of strains of Staphylococcus aureus were found to be sensitive to a concentration of 0.6 μg./cc., and 92 percent to 20 μg./cc. of oleoandomycin.

2. Aqueous concentrations of oleoandomycin were determined following intravenous and subconjunctival injection and by corneal bath. The antibiotic was found to penetrate in high concentration into the aqueous by these methods. Subconjunctival injections were followed by local irritation, edema, and necrosis.

Banting Institute.

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LACRIMAL PROTEINS IN CORRELATION WITH THE SCHIRMER TEST*

FILTER-PAPER ELECTROPHORESIS OF TEARS, TECHNIQUE AND CALCULATIONS FOR RATE, PERCENTAGE OF COMPONENTS, AND PROTEINS

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McEwen's and Kimura's report of the absence of lysozyme in patients with keratoconjunctivitis sicca and Sjøgren's classical articles on the pathology in the clinical pictures in these cases have stimulated interest in this serious problem. Paper electrophoresis, however, was then in its infancy and there were many technical problems to be solved. Even in the past year the Spinco Division of Beckman Instrument Company has made major improvements in electrophoresis paper, in dyes, and in their scanning de-

* From the Stanford University School of Medicine. Presented before the Western Section of the Association for Research in Ophthalmology, November 15, 1957.

vice, the Analytrol. We have changed from the phosphate buffer to barbitol in order to take advantage of the faster methanol soluble dyes.

Present technique

Since June of 1957, three instruments have been used in the filter paper electrophoresis of tears, just as for blood serum (fig. 1).

Duostat

The Duostat is a regulated power supply. It is used to select the type of power regulation desired. We have used five ma. constant current for six hours.

Durrum-type cell

The Durrum-type paper electrophoresis cell consists of a cell vessel, a cell cover, a support stand, and a drying rack together with their auxiliary equipment. The cell accommodates eight paper strips. Electrolyte is stored in two reservoirs in the cell vessel. Each reservoir contains four baffles to prevent electrode products from reaching the paper strips. A port between the two reservoirs makes possible the establishment of equal fluid levels in both reservoirs. Platinum-wire electrodes extend the entire length of each cell to insure that all the strips carry a uniform amount of electricity. The paper strips do not dip directly into the electrolyte. Instead, each strip touches a paper wick, the lower part of which is in the electrolyte. Consequently, at the end of a run, when the individual strip is removed from contact with the wick, no excess electrolyte is left on the paper which might redistribute upon drying. As a result, the pattern on the strip does not become blurred. Cutting or blotting is unnecessary.

The Analytrol

"The primary function of the Analytrol is to evaluate, by means of densitometry, colored or stained material separated electrophoretically, or chromatographically, along a paper strip. The Analytrol may be used, for example, to scan, record, and integrate the optical density of paper strips used in analyzing serum proteins, lipoproteins, amino acids, and proteins of body fluids such as spinal fluid.

"In putting this principal purpose into practice, the Analytrol automatically performs two functions in a single operation. First, it is a calibrated recording densitometer. In fulfilling this function, the Analytrol measures and records some quantity related to the optical density of the sample which is being analyzed by the measuring photocell. As the Analytrol speedily scans the sample, the recording pen automatically draws on a chart a curve of color density versus distance scanned along the paper strip. The second primary function of the Analytrol is as an automatic integrator. Operating simultaneously with the densitometer, a second pen accurately integrates the

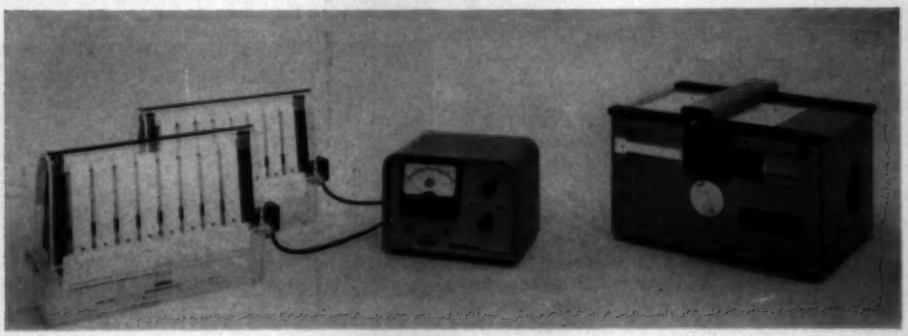

Fig. 1 (Erickson, et al.). Equipment: Durrum cells, Duostat, and Analytrol.

area under the densitometer curve. The integrator pen indicates this area by drawing easily-counted saw-tooth marks. The area thus recorded for each individual component of the sample under analysis is quantitatively related to that particular component.

"When equipped with the B-4 cam and 500 millimicron interference filters, the Analytrol records linearly the concentration of bromphenol blue dye on Schleicher and Schuell 2043A-mgl. paper strips. Thus the integration value or area under each section of the curve is proportional to the amount of total dye bound to the chosen component.

"The following outstanding characteristics are among the new features, the Model RB Analytrol which was additional to those incorporated in the Model RA Analytrol. First, the new Model RB Analytrol includes a new high-gain amplifier, which gives increased sensitivity; this characteristic produces a faster response and enables interference filters to be used. The second new feature, which results from the first, is the use of interference filters instead of absorption filters. Five interference filters, which cover the visible spectrum, are available as accessories. The function performed by these filters is to select the light which passes from the lightsource on to the measuring photocell."

The filter which serves most accurately for the concentration of proteins found in tears is the 600 millimicron filter.

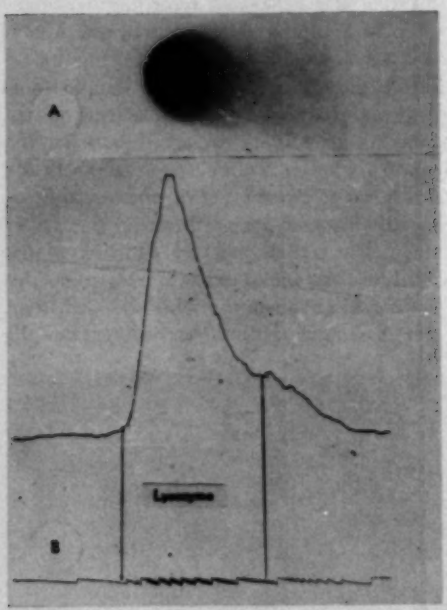

Fig. 2 (Erickson, et al.). Lysozyme control. (A) Lysozyme protein control: 10 lambda, 0.5-percent lysozyme applied on a 17-mm. Schirmer strip. (B) Total protein calculation 75 integration units.

Calculations of protein values (fig. 2)

One of the major proteins of tears is lysozyme. We have, therefore, measured 10 lambda (0.010 ml.) 0.5 percent egg white lysozyme and applied this control on the 17 mm. Schirmer tear strip by pipette. In an electrophoretic (six hours at five ma.) separation, this control moved toward the cathode. The scan strip has three components, the same as human tears, the one toward the farthest left is the highest. A lower hump corresponds to the only lysozyme of monkeys and the protein toward the center overlaps the gamma globulin zone. This probably represents an incomplete separation of the lysozyme from the gamma of egg white.

Lysozyme scan and blood scan (fig. 3)

The blood sample was diluted one to 10 and six lambda of the solution taken for the sample. The scan shows gamma, beta, and alpha 2 globulin zones, and the alpha 1 merging with the albumin.

Schirmer test paper in eye (fig. 4)

It also illustrates the high pH (indicator in colored slide purple) which is present in this particular sample when the rate is low. The normal secretion ought to moisten at least 15 mm. of a strip five mm. wide as measured from the fold at the ciliary margin in five minutes. In order to get the sample centered so that the entire dyed portion passes through the photoelectric cell to be scanned only 17 mm. which absorbs 10

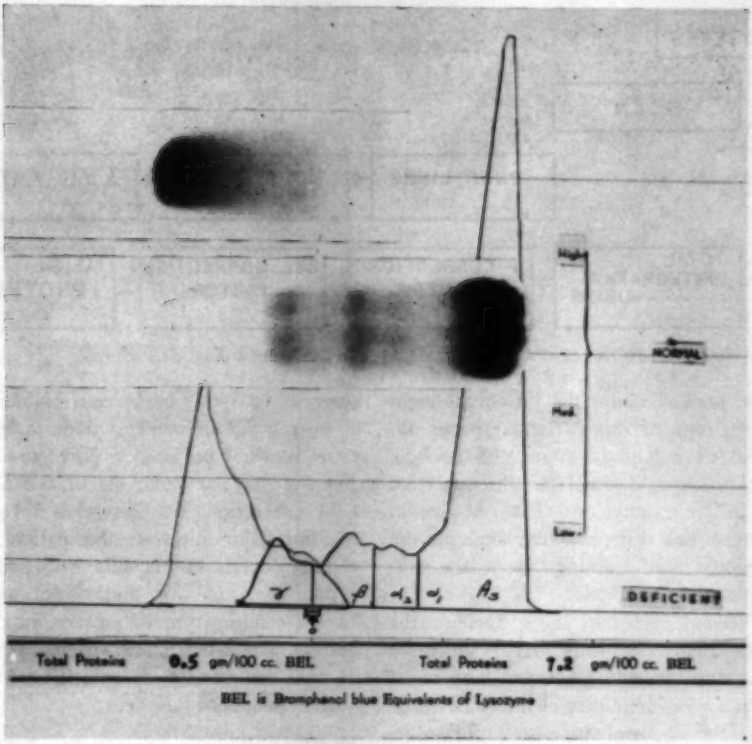

Fig. 3 (Erickson, et al.). Lysozyme scan and blood scan.

lambda is used. If the entire 17 mm. is moistened in four minutes, 2.5 lambda is secreted in one minute.

SCHIRMER TEAR TEST AMPLIFIED (fig. 5)

These two series of steps show: (1) The logic in calculating the rate of flow and (2) the steps taken to make the protein determinations.

Let us, for example, use a three mm. Schirmer test and follow the steps used to arrive at a report on the rate of flow and the percentage of proteins. Three-mm. overflow which is the Schirmer test by definition plus

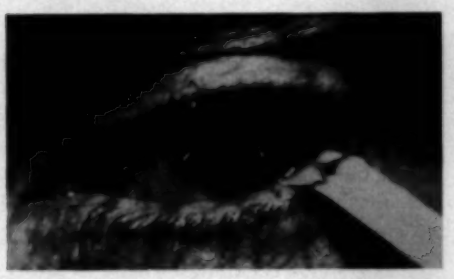

Fig. 4 (Erickson, et al.). The Schirmer test paper in an eye.

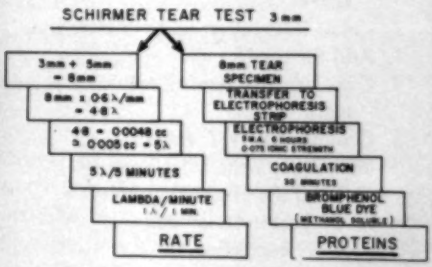

Fig. 5 (Erickson, et al.). Schirmer tear test amplified.

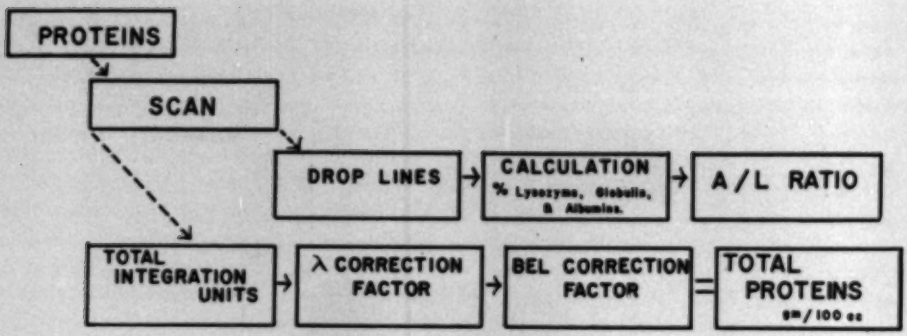

Fig. 6 (Erickson, et al.). Sequence in analyzing a three-mm. scan.

five mm. hooked under the lid equals eight mm. Each mm. of this particular paper absorbs 0.6 of a lambda giving 4.8 lambda. Reduced to three decimal points equals 5.0 lambda in five minutes or 1/1,000 of a cc. in one minute. For this particular example the report would read a simple one in the subnormal column under rate.

The second series of steps reviews the technique. The eight-mm. strip saturated with moisture from the eye and kept moist in a Saran wrap humidity chamber is transferred to the electrophoresis strip. The electrophoresis is carried out as described and the separated proteins are placed in the oven for one-half hour for coagulation and dyed with Bromphenol Blue. The bands of protein or lack of them are then obvious.

SEQUENCE IN ANALYZING A THREE-MM. SCAN (fig. 6)

1. Draw in lines to separate mobility zones
2. Count integration units in each zone
3. Add the total
4. Calculate the percent of each component.
5. Compute the A/L ratio.
6. To calculate the total proteins
   a. Total the integration units
   b. Correct to 10 lambda
   c. BEL correction.

In this particular example we have three-percent lysozyme, 67-percent globulins, and 30-percent albumins. The albumin/lysozyme ratio is 10. The lambda correction is two and for this particular set up 0.0067 is the BEL correction. The answer is 1.4 gm./100 cc. For your interest, the patient was a young woman in her 30s who had had a hypophysectomy for metastatic carcinoma. This ascending type of pattern, however, is not characteristic of any special disease and is very common in chronic illness and for normal people in fatigue.

FROM SCHIRMER TO RATE CONVERSION (table 1)

If, for instance, the patient moistened the entire 17 mm. in one-half minute, he would have secreted 20 lambda per minute. Two and one-half lambda per minute is the accepted Schirmer level of normal. Many of the men in our industrial studies had Schirmer tests which were below this level especially at the end of their working day.

NORMAL TEAR PROTEINS AND SCAN (fig. 7)

Figure 7 shows the three bands of dyed concentration in the normal tear. The scan below shows the three major peaks. The rate of 10 lambda per minute, 22-percent lysozyme, 60-percent globulins, and 18-percent albumins with a total protein of 2.0 gm./100 cc.

ASCENDING PATTERN (fig. 8)

Figure 8 shows the tear protein for Mr.

TABLE 1

CONVERSION TABLE FOR THE 17 MM. SCHIRMER PAPERS TO RATE

(Based on 0.6 lambda per mm.)

	Grade*	Mm. of Schirmer (mm.)	Minutes	Seconds	Rate Lambda/Min.
Psychic tearing	10	17	—	—	—
Above normal	9	17	0	15	40
Normal	8	17	0	30	20
		17	0	45	15
	7	17	1	0	10
		17	1	7	9
		17	1	15	8
		17	1	26	7
		17	1	40	6
		17	2	0	5
	6	17	2	30	4
		17	2	51	3.5
		17	3	2	3.3
		17	3	20	3.0
Limit range	5	17	3	34	2.8
		17	3	51	2.6
		17	4	0	2.5
	4	17	4	10	2.4
		17	4	21	2.3
		17	4	33	2.2
		17	4	46	2.1
		17	5	0	2.0
Subnormal	3	16	5	0	1.9
		15	5	0	1.8
		14	5	0	1.6
		13	5	0	1.5
	2	12	5	0	1.4
		11	5	0	1.3
		10	5	0	1.2
		9	5	0	1.0
	1	8	5	0	0.9
		7	5	0	0.8
		6	5	0	0.7
		5	5	0	0.6
		4	5	0	0.4

* For purposes of research classification the rate grades from our clinical experience is as follows:

10. Psychic tearing
9. Above normal
8. High } Normal
7. Medium } Normal
6. Low } Normal
5. Upper } Limit range
4. Lower } Limit range
3. Minimal } Subnormal
2. Moderate } Subnormal
1. Marked } Subnormal

A. B., aged 67 years, who came in with the complaint, "My left eye is killing me." Upon examination, he had marked punctate staining on the conjunctiva and the cornea particularly upon the left eye which gave him so much discomfort. With the lipotropic drugs, which improved the lysozyme secretions and 0.5-percent methyl cellulose for artificial tears, he has remained fairly comfortable, but about a week before this episode, he had an upper respiratory infection The tear pattern in the right is normal with

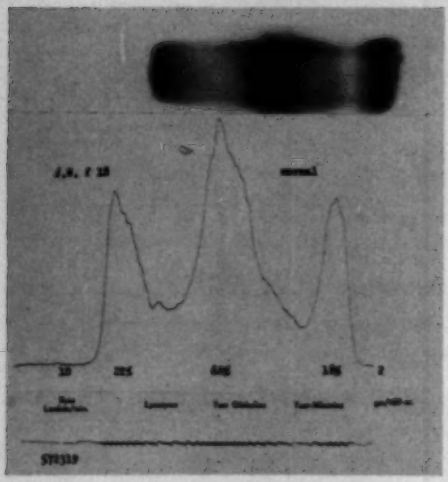

Fig. 7 (Erickson, et al.). Normal tear proteins and scan.

an albumin/lysozyme ratio of 1.5. The ratio in the left specimen, however, is more than eight. It has been our experience that when the albumin/lysozyme ratio is high, the pH is high, the rose bengal punctate staining test is usually positive, and, as with this patient, relief is urgently requested.

Ascending pattern associated with conjunctivitis and narrow-angle glaucoma (fig. 9)

Figure 9 shows the protein and scan from a glaucoma patient whose eye was very red and uncomfortable. Because she had a very narrow angle one would certainly not suspect that her major problem was a conjunctivitis. Her eye was red and uncomfortable with a pressure of 36 mm. Hg (Schiøtz) when this specimen was taken. With corti-

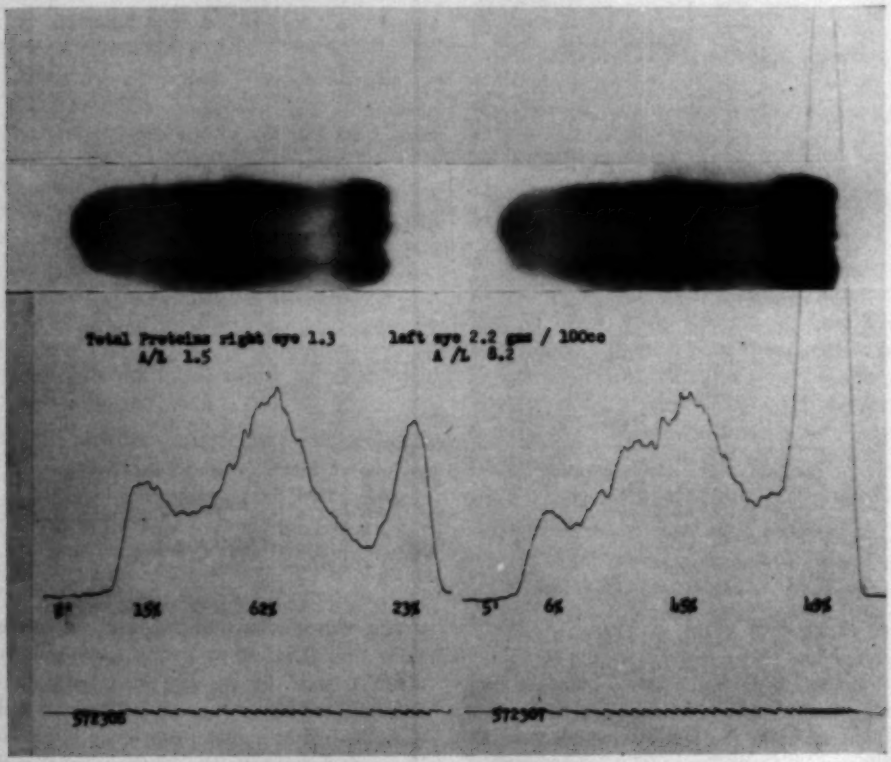

Fig. 8 (Erickson, et al.). Ascending pattern. A. B., male, aged 67 years, reported "My left eye is killing me." He showed punctate staining of conjunctiva and cornea.

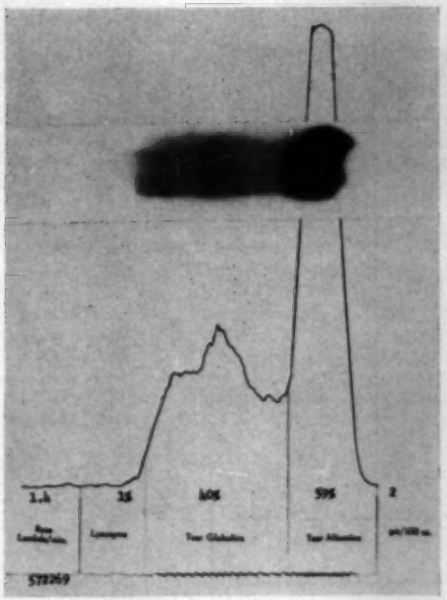

Fig. 9 (Erickson, et al.). Ascending pattern associated with conjunctivitis and narrow-angle glaucoma. F. H., female, aged 68 years, narrow-angle glaucoma.

sone and antibiotics locally and lipotropic drugs by mouth, her eye cleared and the pressure came down to 22 mm. Hg under the same miotic.

Fatigue patterns (fig. 10)

Figure 10 shows the proteins from another patient whose chief complaint was burning of the eyes. The proteins to the left was taken at 8:00 A.M. from one eye and the two at the right were taken when he came off work at 5:00 P.M. The rate went from 2.5 lambda to 0.6 lambda per minute and the albumin/lysozyme ratio changed from a normal 1.5 to 5.2. Many of the 1,200 industrial determinations we made did, in fact, show with fatigue a decrease in the rate and a disturbed protein balance.

Fibrocystic pattern (fig. 11)

Figure 11 shows proteins from a child with a diagnosed fibrocystic disease. There is an ascending pattern with a high albumin/lysozyme ratio. An ascending pattern is quite common with a subnormal rate but an ascending pattern with a normal rate seems to be quite characteristic of this disease.

Conclusion

In conclusion we can say that the proteins are essential to a viscous tear film maintaining a normal pH. As a general rule lysozyme is the first protein to decrease as the rate of flow decreases.

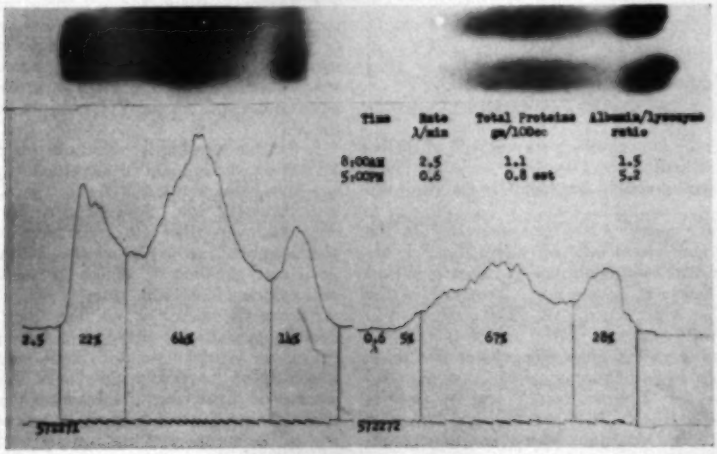

Fig. 10 (Erickson, et al.). Fatigue patterns. P. D., male, aged 52 years, reported "eyes burn."

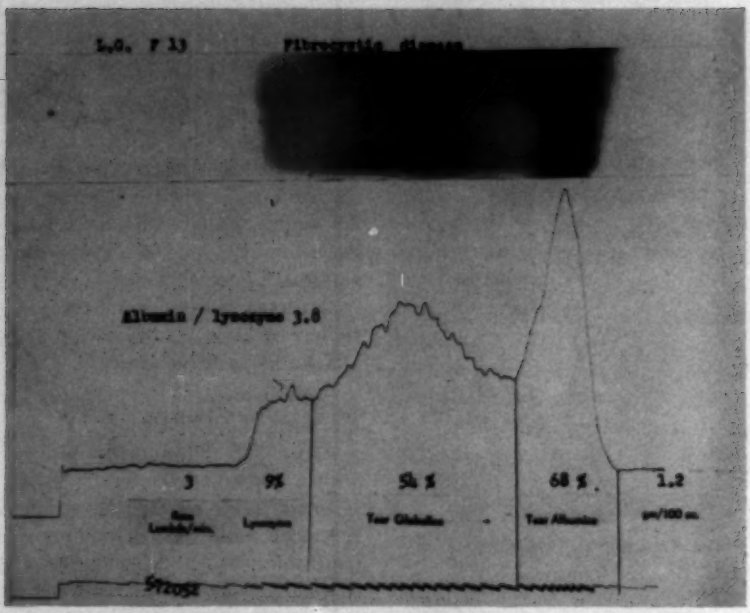

Fig. 11 (Erickson, et al.). Fibrocystic pattern. L. G., female, aged 13 years, showed fibrocystic disease.

The protein balance is usually further upset by a simultaneous rise in albumins. It is at this stage when patients complain of burning and discomfort. As the rate approaches *one*, all three lacrimal proteins begin to disappear and a sicca pattern results.

1990 24th Avenue (16).

Discussion

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Dr. Erickson is uncovering a great deal of undiscovered information about lacrimal secretion, much of which will probably be found to be clinically useful.

I have for many years been interested in the pathology and physiology of lacrimation. I attempted to read everything that had been published about it; however, I was more interested in the quantity, that is the wet eye vs. the dry eye, than in the quality of the tears.

The chief contribution to this subject was made by Schirmer in his "Microscopisch Anatomie und Physiologie der Thranenorgani," and in *Graefe-Saemisch Handbuch* (1904). Campos and Lutz also contributed their findings. There were many observations but the following facts were generally agreed upon:

1. The normal daily lacrimal secretion is from 0.3 to 0.5 cc.

2. The principal sources are the conjunctival glands of Krause and Sappey.

3. The lacrimal gland provides an accessory supply, utilized to remove foreign bodies, remove secretion, provide lysozyme, and so forth.

4. The nerve supply to the lacrimal gland is from the lacrimal branch of the first division of the V nerve, the zygotemporal of the II, the subcutaneous malae or other branches which come from the sphenopalatine ganglion, via the great superficial petrosal nerve from the VII and join the two from the V.

5. Psychic weeping is entirely dependent upon the VII (this was brought to my attention many years ago by a patient with a VII nerve paralysis from a basal fracture who observed that she cried with only one eye. Other observers have reported this phenomenon. Extirpation of the sphenopalatine or geniculate ganglion or section of the vidian nerve also produces this result.

Schirmer's studies proved the three sources of nerve supply to the gland, and his technique included an irritation of the nasal mucosa with a cotton-tipped applicator in cases in which the amount of moistening of the blotter paper was less than 1.5 mm. in two minutes. He believed this to add to the accuracy of the test and to differentiate

between the stimulation from the first and second division of the V nerve, when the conjunctiva had been cocainized. He also stimulated secretion by flashing bright lights on the retina.

Dr. Erickson, in her lysozyme and protein type determinations, has gone far beyond Schirmer's chemical determinations of tear constituents. She has also added significance to Sjøgren's observations.

The matter of psychic weeping is an interesting aspect of this subject; it is not present in infants or experimental animals. Electric stimulation of the VII nerve in apes does produce contraction of the facial muscles and slight tearing, as occurs in humans, on laughing or crying. However, the nerve connections between the sphenopalatine ganglion and the lacrimal nerve and gland have not been worked out. Studies to differentiate the quality of tears under normal conditions and after electric or psychic stimulation might yield different results, and would be an interesting continuation of Dr. Erickson's work. She is to be highly commended for her zeal and patience in acquiring data and it is hoped that she may continue.

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CORNEAL ULCERS PRODUCED BY CELL-FREE EXTRACTS OF PSEUDOMONAS AERUGINOSA*

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New Orleans, Louisiana

Introduction

Since Pseudomonas aeruginosa is not a highly invasive agent, especially when located in normal tissue, infections caused by this organism are often correlated with the general state of health of the individual. In ocular infections, however, a different situation exists, especially where corneal involvement occurs. The organisms are introduced through a break in the tissue where they then grow and produce extensive ocular damage.

For the most part, previous studies by other investigators have dealt with the effect of antibiotics on pseudomonas corneal infections.[1-3] Spencer[1] lists a rather complete bibliography concerning pseudomonas ocular infections. This literature records chiefly the history, case progress reports, and possible treatment of the disease. Our investigations primarily involve studies concerning certain factors elaborated by Pseudomonas aeruginosa which we believe play an important role in the destruction of certain ocular tissues.

Experimental studies

A number of strains of Pseudomonas aeruginosa were collected and maintained in stock culture on a Difco Tryptose Blood Agar base medium, in the absence of blood.

Preliminary to the studies to be reported, experiments were designed to ascertain the degree of pathogenicity or virulence of all strains for ocular tissue. For this purpose 24-hour broth cultures were used. Healthy rabbits were inoculated intracorneally with 0.02 ml. of the bacterial culture while control inoculations were made with 0.02 ml. heat killed 24-hour broth cultures. In each instance, the inoculum left an opaque bleb in the cornea measuring approximately two mm. in diameter but in the control eyes these opacities disappeared within the first 24 hours. Corneas inoculated with the several

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living strains of pseudomonas exhibited various degrees of damage. Some of the strains were apparently completely avirulent showing no manifestations of corneal infection whereas several strains produced rapidly advancing corneal disease accompanied by marked tissue destruction.

Subsequent experiments herein reported were made with a strain of pseudomonas isolated from a severe corneal infection. This organism proved to be the most virulent of any strain tested by the rabbit cornea assay technique. Figure 1A is a photograph of a rabbit's cornea taken immediately following inoculation while Figure 1B shows the same eye 24 hours later. During the 24-hour test interval the conjunctiva became injected, a greenish-yellow mucopurulent discharge appeared, and a rapidly advancing corneal ulcer developed. Heat killed standard suspensions of the same organism introduced intracorneally caused no permanent damage.

After selection of this strain of Pseudomonas aeruginosa, the bacteria were cultured for 18 hours in trypticase broth media. Bacteria were removed by centrifugation, washed twice in sterile saline, then resuspended in saline to the same turbidity as that given by the 18-hour culture, when measured in a spectrophotometer using a wave length of 660 mμ. Intracorneal inoculations using 0.02 ml. of the washed saline suspension produced the same type of corneal damage as was observed for the 24-hour broth culture. Pseudomonas aeruginosa organisms were reisolated from the ulcers on blood agar plates from both trypticase broth culture and saline suspension test samples.

The next study was made with a cell-free broth preparation obtained following centrifugation of the 18-hour trypticase broth culture. This broth specimen was sterilized by filtration and used for intracorneal inoculations in 0.02 ml. aliquotes. Control inoculations were made with 0.02 ml. quantities of sterile uninoculated trypticase broth medium. Corneal ulceration was produced by the culture broth from which pseudomonas had first grown and was later removed from the fluid while eyes inoculated with trypticase broth which had never supported bacterial growth showed no ulceration. Pseudomonas could not be cultured from eyes of the test or control rabbits.

Due to the appearance of the active infection, as well as the damage resulting from the filter sterilized culture broth a toxin, proteolytic enzyme (or enzymes), or a combination of these factors were thought to play an important role in the extensive destruction of these corneas. Work was, therefore, initiated to study the possible relation-

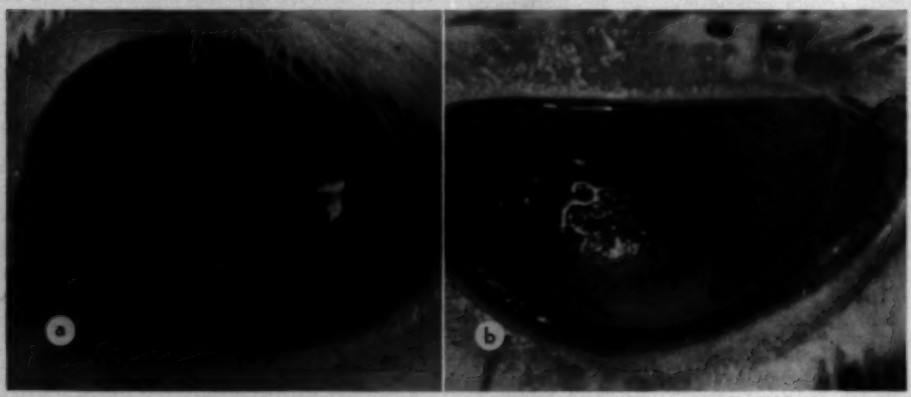

Fig. 1 (Fisher and Allen). Photographs taken immediately after (a) and 24 hours following (b) intracorneal inoculation of a trypticase broth culture of the Pseudomonas aeruginosa strain used in this work.

ship of pseudomonas protease(s) to corneal damage.

To determine the effect of various culture fluids on corneal tissue in terms of proteolytic activity experiments were carried out with three specimens; in the first, the whole trypticase broth culture which had incubated 18 hours was used, the second sample was composed of an autolysate of washed bacterial cells removed from the 18-hour trypticase broth culture, while the third test material consisted of supernatant fluid removed from the 18-hour trypticase broth cultures.

Proteolytic activity was followed by means of a colorimetric procedure, which depends on the release of colored nontrichloroacetic acid precipitable products from a diazotized protein by the protease enzyme. The method of protein diazotization was essentially that of Tomarelli[4]; the only modifications of this method involved the amounts of reagents and the proteins tested.

Conditions of the assay procedure were established as a result of data obtained from kinetic studies involving the proteases of pseudomonas.[5] The incubation mixture was composed of 0.5 ml. of the protease, 0.5 ml. Tris buffer* 0.2 M pH 7.5, and 0.5 ml. diazotized casein (or diazotized protein), 5.0 mg. per ml. After incubating the mixture for six minutes at 37°C., the reaction was stopped and the undigested protein precipitated with 3.5 ml. of five-percent trichloroacetic acid. The acidified mixture was then filtered to remove the precipitated proteins and the color was intensified by adding 2.5 ml. of 0.5 N NaOH to 2.5 ml. of the filtrate. The optical density was read at 420 mμ wave length in the Coleman Universal Spectrophotometer.

A unit of proteolytic activity defined on the basis of casein digestion is as follows: one unit is equivalent to the release of one μg. of diazocasein following digestion using the procedure described above. The recorded spectrophotometric readings were compared to a standard curve of known values in μg. units of diazocasein.

The standard curve was prepared by accurately weighing a sample of dried diazocasein, dissolving it in distilled water, and preparing a series of dilutions, thus obtaining solutions of different known concentrations. The diluted samples were read colorimetrically using undigested specimens without following the trichloroacetic acid precipitation procedure. To prepare the standard curve optical density values were plotted against the amount of diazocasein present.

Washed cell autolysate, cell-free culture supernatant fluids, and 18-hour trypticase broth cultures were shown to have proteolytic activity as measured by the above procedure (table 1). The proteolytic enzyme fraction was then partially purified by precipitation from the cell-free culture supernatant using ammonium sulfate and ethyl alcohol as protein precipitants.[6] Further evi-

* Tris (hydroxymethyl) aminomethane.

TABLE 1

PROTEOLYTIC ACTIVITY

Sample	Diazocasein Digested (μg.)	Protein in Sample Assayed (mg.)	Volume of Material Assayed (ml.)
Pseudomonas broth culture	170	—	0.1
Pseudomonas washed cell autolysate	360	0.25	0.1
Cell-free culture supernatant fluids	140	1.0	0.1
Cell-free culture supernatant protein precipitant	730	0.35	0.1
Pseudomonas synthetic culture	0	—	0.1
Crude trypticase cell extract	1640	2.4	0.1
Crude synthetic cell extract	0	2.6	0.1
Cell extract purified proteolytic fraction	1540	0.45	0.1
Trypsin	1160	1.0	0.1

Fig. 2 (Fisher and Allen). Photograph taken 12 hours following intracorneal inoculation of protease concentrated from the cell-free supernatant of 18-hours trypticase broth culture of pseudomonas.

dence recorded in Table 1 indicates the partial purification procedure considerably enhanced the proteolytic activity of these fractions, since the ratio of activity of the enzyme to the quantity of protein per fraction was increased. The protein content was measured by the method of Stadtman.[7] When this material was inoculated intracorneally into rabbits, severe corneal damage was observed. Figure 2 is a photograph, taken 12 hours following inoculation, which shows perforation of the cornea with extrusion of intraocular substances. When the test material was heated to denature the proteins, all activity was lost—both proteolytic properties and the ability to produce corneal damage.

The next experiment was designed to ascertain the influence of the growth medium on elaboration of corneal destructive substances by the pseudomonas organism. Pseudomonas was cultured in a synthetic medium* for 18 hours at 37°C. Very little proteolytic activity was obtained when the strain of pseudomonas was grown in a synthetic medium and appropriate fractions were tested in rabbit corneas. Of course, the virulent strains of pseudomonas, when grown under such conditions, were still able to produce corneal ulcers because the bacteria multiply in corneal tissue. When cell-free broth from such a culture was introduced into the eye, corneal damage did not occur, again suggesting a correlation between corneal destruction and protease activity.

Since whole washed cells grown in a trypticase broth medium have proteolytic activity, it was decided to disrupt the cells and fractionate the cellular material in an attempt to obtain the corneal destructive factor in pure form. Packed cells from a trypticase broth culture were mixed with an abrasive, Alcoa alumina A301, and ground in a mortar in order to disrupt cells and release the internal cellular constituents. The soluble proteins were extracted with saline while the remaining whole cells, cell fragments, and the abrasive material were removed from the suspension by centrifugation.

This treatment yielded a clear amber solution composed of soluble cellular substances which were primarily protein in nature. When this soluble protein fraction was inoculated intracorneally into rabbits severe corneal damage was realized (fig. 3). The whole cornea appeared to be destroyed or liquefied with perforation and extrusion of intraocular material including lens tissue, which occurred as a result of proteolytic destruction of ocular structures.

Repeated attempts to demonstrate pseudomonas by either culture methods or by microscopic techniques were unsuccessful. The ocular damage was, therefore, due to soluble factors in the extract and was not an immediate result of the activity of living organisms. Extracts, tested for their proteolytic capacities, were found to contain a high level of activity (table 1) as measured by the protein degradation procedure mentioned earlier.

* Composition of the synthetic medium is as follows: Glucose, 10 g.; KH_2PO_4, 3g.; K_2HPO_4, 7g.; NH_4Cl, 0.1 g.; $NaNO_3$, 1 g.; $MgSO_4 \cdot 7H_2O$, 0.1 g.; $FeSO_4 \cdot H_2O$, 0.001 g.; $CaCl_2$, 0.01 g.; distilled water one liter.

An extract prepared from a washed pseudomonas cell suspension grown in synthetic medium was partially purified by means of protein precipitants. This extract contained very little activity. The proteolytic enzyme content was almost nil as measured by our assay procedure (table 1). Intracorneal inoculation of the extract produced very little effect as shown in Figure 4. This picture was taken 24 hours following intracorneal inoculation of the extract. It may be noted that 24 hours later very light corneal opacity remained from the bleb produced at the site of inoculation. It, therefore, seems reasonable to infer from these observations that growth of the organisms in the presence of organic materials, probably proteins, is essential for elaboration of proteolytic enzymes which play an active role in corneal destruction.

Several protein fractions were obtained when extracts prepared from cells grown in trypticase broth medium were fractionated according to previously described methods. One of the fractions which did not contain proteolytic activity was likewise unable to produce corneal damage. A relatively high degree of proteolytic enzyme purification was

Fig. 3 (Fisher and Allen). Photograph taken 12 hours following intracorneal inoculation of an extract prepared from washed cells grown in a trypticase broth medium.

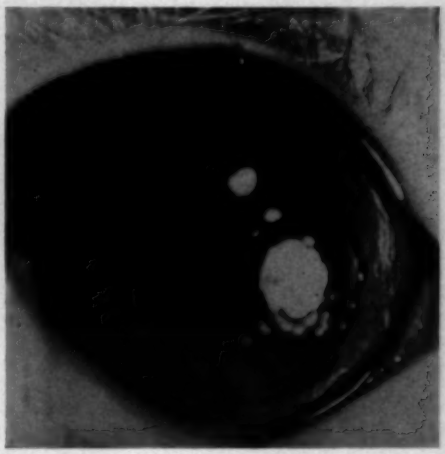

Fig. 4 (Fisher and Allen). Photograph taken 24 hours following intracorneal inoculation of cell extract prepared from washed cells grown in a synthetic medium.

obtained from one of the above fractions. In this instance the ratio of proteolytic activity to protein content was much higher in the purified fraction as compared with the crude extract. The data are recorded in Table 1. When this purified fraction was inoculated intracorneally into rabbits a very severe reaction was demonstrated, resulting in a marked degree of corneal damage concurrent with destruction of other ocular structures (fig. 5).

Since the correlation between proteolytic activity and corneal damage does exist in pseudomonas infections, it was decided to test certain other commercially available proteolytic enzymes in the same way. A two times crystallized preparation of trypsin (Nutritional Biochemicals) was obtained which was diluted to contain the same amount of protein as that present in the pseudomonas extract. The trypsin solution was then checked for its ability to cause ocular damage. When inoculated intracorneally, trypsin produced a rather mild reaction within a 24-hour period as was observed by slight corneal opacity but later there was very definite clearing in the center of the inoculated eyes. When proteolytic activity of the

Fig. 5 (Fisher and Allen). Photograph taken 12 hours following intracorneal inoculation of a purified protease fraction prepared from a cell extract of trypticase broth grown cells.

trypsin was tested by the described colorimetric procedure, a fair degree of activity was measured; again these values are recorded in Table 1. Thus, in the case of trypsin, proteolytic activity of the enzyme is not correlated with corneal destruction while the protease from pseudomonas definitely destroyed ocular structures. There are also other chemical features which suggest differences between the pseudomonas enzyme as compared to trypsin.[5]

Discussion

One of the characteristic features of a pseudomonas ocular infection is the extensive corneal damage. This work suggests that possibly a protease is elaborated by Pseudomonas aeruginosa which then causes corneal destruction.

It was observed that a corneal destructive material was released by the organism into the medium and this substance had the ability to cause ocular damage. This action is correlated with protease activity of the culture. Neither corneal destructive factor nor protease enzyme activity could be detected when the organism was grown in a synthetic medium. These findings suggest that the protease is an adaptive enzyme requiring the presence of an appropriate protein substrate before the enzyme is operative.

In the case of whole cells, the growth medium did not influence the virulence of the organism since when these organisms were introduced into the cornea, proteins of the cornea were sufficient to initiate production of the protease.

Crude cell fractions which contained large amounts of proteolytic activity also caused very extensive corneal damage. When the crude fraction was separated into various protein subfractions, the corneal destructive activity followed the protease activity suggesting that they are the same. In purification procedures, where the protease activity was increased per unit of remaining protein, the corneal destructive activity also increased in terms of the severity of ocular damaging ability.

Not all proteases have corneal destructive properties. Trypsin, the one protease used in these studies which was not of bacterial origin, initiated a rather mild reaction as compared to that of the pseudomonas enzyme. This observation is not out of line since it is well known that enzymes are generally specific for their substrates in one manner or another. The same fact holds true for known proteases which have specific peptide linkage or amino acid residue requirements in order to be active. Further studies regarding enzymatic kinetics of the pseudomonas protease are in progress in an attempt to understand the reaction requirements and the mode of action of this system. A comparison of proteases other than trypsin will be made with the pseudomonas enzyme to note differential properties.

Once the mechanism of this protease reaction is understood the data may have a practical therapeutic application since an enzyme inhibitor of some type may be used in the prevention of ocular destruction. Antibiotics combat only living cells and not ex-

tracellular products elaborated by bacteria. Possibly an anti-enzyme inhibitor could effectively inactivate the pseudomonas protease thereby preventing corneal destruction in pseudomonas infections.

Summary

Strains of Pseudomonas aeruginosa have been obtained which exhibit varying degrees of virulence ranging from those initiating no corneal damage to those producing marked corneal damage.

A correlation has been demonstrated between the proteolytic capacity of the organisms and the corneal ulceration observed. The protease, obtained in cell-free preparation, caused severe damage to ocular structures but the pseudomonas proteolytic enzyme factor whose activity is responsible for corneal destruction is not an inherent capacity of all proteases.

Kinetic studies are in progress to characterize the pseudomonas protease enzymes described above. Possible immunologic considerations will also be pursued.

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ELECTRON MICROSCOPIC STUDIES OF THE HUMAN EYE*

I. Preliminary investigations of the trabeculas

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In recent years interest has been focused on the structures of the angle of the anterior chamber as probable sites of changes responsible for open angle glaucoma. To date, however, no electron microscopic studies of human trabecular meshwork have been recorded in the literature. In the present study, thin sections of this structure have been subjected to electron microscopic examination. The preliminary results are reported herewith.

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Materials and Methods

One of the major requirements of the electron microscopist in the study of human tissues is the acquisition of specimens so fresh that no postmortem autolytic changes have yet occurred. Rhodin[1] conducted an experimental analysis of the changes that take place in cell structures at various intervals after the death of an animal. He found no apparent change at five minutes, and only slight swelling of the inner membranes of the mitochondria at 15 minutes. At 60

minutes, however, there were marked swelling of the mitochondria, coarse granulation of the cytoplasmic ground substance, and vacuolization of the nucleus.

Of the four eyes sectioned for the purposes of the present study, one was fixed one minute after enucleation, two were fixed 18 minutes after death, and one was fixed 45 minutes after death.

The fixative most commonly used in electron microscopy is one-percent buffered osmic acid (pH 7.4). It is not only an excellent cytoplasmic fixative but an "electron stain" with an affinity for such lipoid structures as nuclear and cytoplasmic membranes. Osmic acid penetrates slowly (the rate varying with the type of tissue) so that it is wise to restrict the size of the specimens to one mm. or less. Tissues fixed with osmic acid turn jet black in the first alcohol bath. Since this obscures the anatomic landmarks, it is important to cut the specimens in definite shapes to indicate their orientation. They often change position during polymerization in the gelatin capsule, however, and it is difficult to orient them with the light microscope; osmic-fixed tissues take ordinary stains poorly and the histochemical reactions are altered.

In this study the eyes were fixed in one of two ways:

1. A 30-gauge needle was inserted into the anterior chamber and the aqueous was replaced with fixative. The eye was then gently perfused with the fixative for several minutes. It was then bisected equatorially and the anterior segment placed in a beaker of osmic acid for fixation from two to four hours.

2. The eye was bisected in the anterior-posterior meridian. One-half was placed in osmic acid and the other half in formalin for histologic studies with the light microscope. The first of these methods has yielded better results, possibly because perfusion of the fixative effects quicker penetration of the trabeculas. In this paper, specimens fixed only by method No. 1 are described.

After fixation the specimen was carefully dissected under a dissecting microscope with new razor blades and No. 67 Beaver blades. When a special orientation was desired, specimens 1.0 by 1.0 by 3.0 mm. were cut and a suture of 6-0 black silk was passed through one end. Numerous specimens of trabeculas could be cut from one eye.

The dissected specimen was then rinsed in distilled water, dehydrated in alcohols, infiltrated with n-butyl methacrylate according to the schedule of Farquhar,[2] and embedded in a gelatin capsule in a solution of n-butyl methacrylate with the catalyst. The specimen was placed in the capsule with the proper face at the bottom, and the resin was polymerized at 45°C. for 18 to 25 hours. If the block turned during polymerization, it was re-embedded, the suture serving as a guide.

Sections were cut with the Porter-Blum microtome. With the glass knife of this instrument it is possible to obtain slices approximately 0.02 μ thick. These were picked up on copper grids covered with a film of Formvar. The sections to be examined were selected for thinness on the basis of interference colors and were stored in a desiccator until viewed. Occasional "thick" (1.0 μ) sections were placed on glass slides and stained for 30 minutes or longer with hematoxylin to check the orientation.

Electron micrography was taken with an RCA-EMU-2 microscope fitted with a 25 to 30 μ objective aperture. Those reproduced for presentation with this report were taken from four different specimens which may be described as follows:

Specimen 490 was the eye of a 44-year-old female who died of cirrhosis of the liver. The eye was fixed 45 minutes after death. In view of the undue lapse of time between death and fixation, the only photography presented from this specimen is one of typical collagen of 640 Å periodicity from the sclera.

Specimen 523 was the eye of a 48-year-old female whose orbit had been exenterated

for malignant melanoma. The eye was fixed one minute after enucleation.

Specimens 610 and 609 were the eyes of a 67-year-old male who died of carcinoma of the bladder. They were fixed 18 minutes after death.

Observations and Discussion

The montage (fig. 1) represents an area of trabecular tissue approximately 70μ by 65 μ, bordering Schlemm's canal. This montage shows the relationship of the individual trabecular fibers, the endothelial cells, the open spaces of the meshwork, and the canal of Schlemm.

The classical description of a corneoscleral trabecular fiber given by Salzmann[3] is as follows:

"The individual trabecula consists of four elements. Its foundation is formed by a thick non-nucleated bundle of collagenous fibrillae; the fibrillae run parallel to the long axis of the trabecula, therefore, mainly circular. This connective tissue is supported along its surface by relatively thick elastic fibers; they course in nearly the same direction as the collagenous fibrillae. . . . A glass membrane succeeds the elastic fibers . . . and possesses all the tinctorial and morphologic peculiarities of Descemet's membrane. The whole is finally covered by an endothelium which, as usual, shows no cell borders, so one can recognize the individual cells only through the prominent oval nuclei."

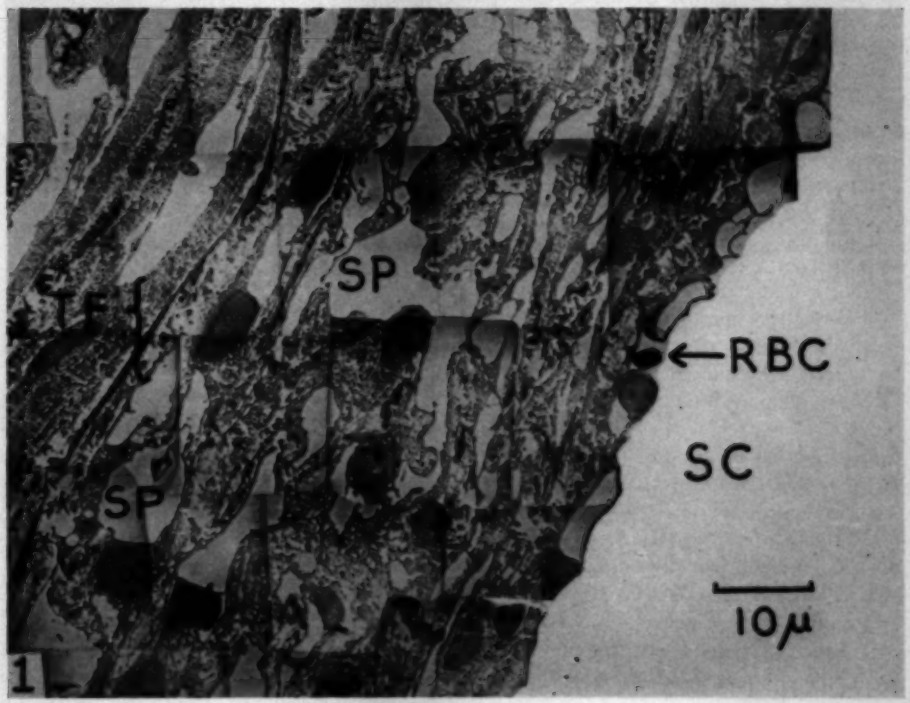

Fig. 1 (Garron, et al.). Montage of a small portion of trabecular meshwork measuring approximately 70 μ × 65 μ near the scleral spur and bordering Schlemm's canal. (SC) The endothelial cells lining the wall of the canal show giant cytoplasmic vacuoles. A portion of a red blood cell (RBC) may be seen between two endothelial cells. In the trabecular meshwork, several endothelial nuclei are seen, some showing nucleoli. A trabecular fiber (TF) on the left has been cut longitudinally through its collagen core. The trabecular spaces (SP) are bound by cytoplasm which covers the trabecular fibers. (×2,000.)

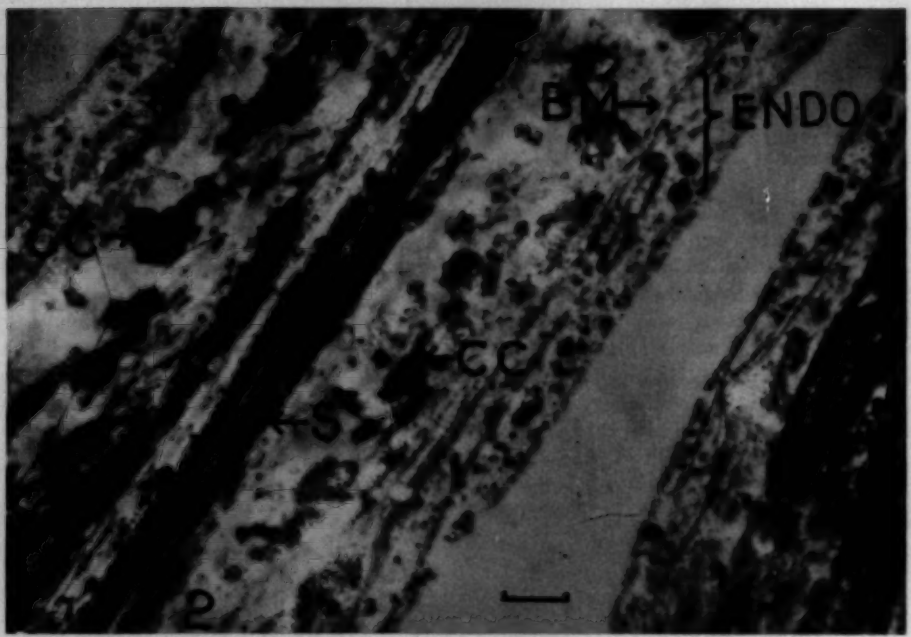

Fig. 2 (Garron, et al.). A longitudinal section through the center of a trabecular fiber. The outermost layer is the cytoplasm (ENDO) of an endothelial cell. Beneath this is the basement membrane (BM) followed by a wide matrix of light density in which are scattered structures having a periodicity of about 1,000 Å (CC). Through the center of the band is a core of tightly packed collagen fibrils having a periodicity of approximately 640 Å. On either side of this core there is a layer which also has a periodicity of about 1,000 Å (S). Overlapping cytoplasm of adjacent endothelial cells may be seen (×10,000.)

The electron microscope revealed a more detailed, orderly structure than has been seen with the light microscope, but the classical four-component arrangement of a trabecular fiber as described above was confirmed only so far as the collagen core and outer endothelial covering were concerned. The existence of "relatively thick elastic fibers" and a Descemetlike membrane could not be verified. Our findings will be discussed under headings corresponding to Salzmann's four principal components, and in a special paragraph on Schelmm's canal.

Collagen core

A longitudinal section through a trabecular fiber (fig. 2) showed the central collagenous core to be a bundle of fibrils so tightly packed together as to obscure fibrillar detail. Their periodicity, approximately 640 Å, was typical of the banding of ordinary collagen when viewed with the electron microscope (fig. 3).

The collagen core varied in width and divided into X- and Y-shaped figures to form the core of other trabecular bands in the meshwork. The core appeared to be enveloped by a sheath of material 800-1,300 Å thick with the fibers showing a periodicity of approximately 1,000 Å (fig. 4).

Elastic fibers

Structures resembling elastic fibers were found distributed at random throughout the trabecular meshwork. They were apparently inconstant components of the corneoscleral trabeculas and were in no case found adjacent to the central collagen core. They were covered by a sheath of material with 1,000 Å

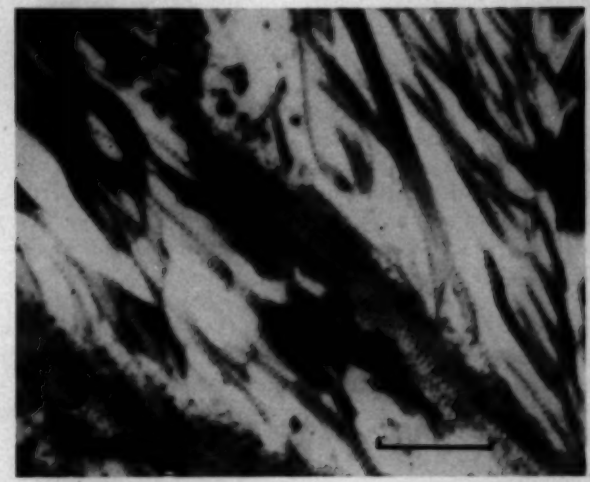

Fig. 3 (Garron, et al.). An ultrathin section of sclera showing the 640 Å banding of typical collagen. (×20,000.)

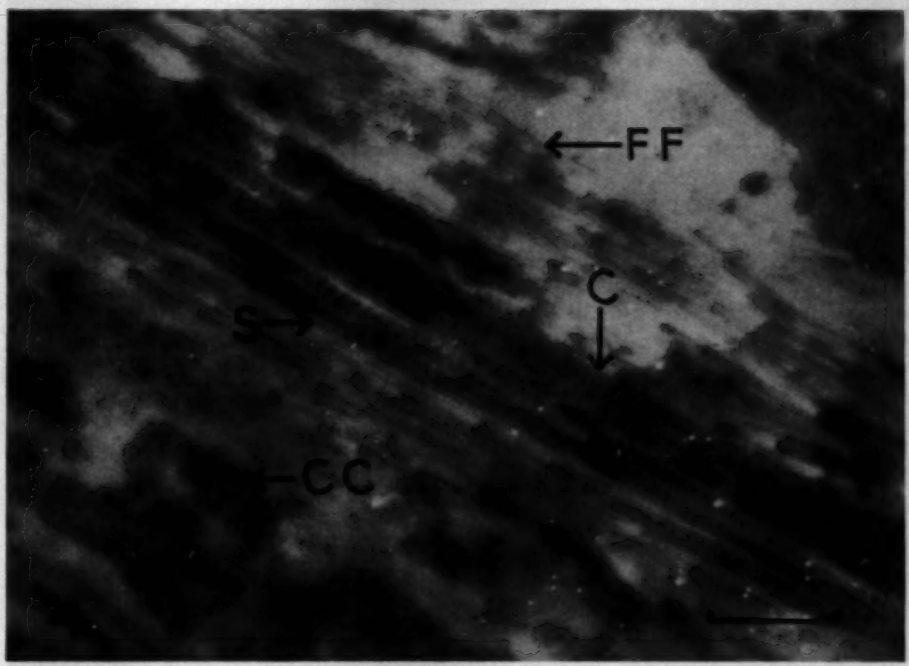

Fig. 4 (Garron, et al.). A longitudinal section through a trabecular fiber (cut through the margin of collagen core). The sheath of fibers having 1,000 Å periodicity (S) can be seen adjacent to some fibers of 640 Å periodicity (C). Fine fibrils (FF) having no visible periodicity are scattered in the matrix along with the irregular structures of 1,000 Å period (CC). (×20,000.)

periodicity, apparently identical to the material covering the collagen core (fig. 5), and were often found beneath the basement membrane of the endothelium.

The collagen core and its sheath were surrounded consistently by a homogeneous matrix of light density in which there was a scattering of very fine fibrils and irregular structures with a periodicity in the order of 1,000 Å. The homogeneous area between the collagen core and the basement membrane of the endothelial cells may be either lightly or densely packed with this 1,000 Å period material. The aggregates of this material appeared in a variety of configurations: oval, crescentic, round, or rod-shaped (fig. 6). If this unusual substance is a fiber, one must presume that it has a twisting, tortuous course through the matrix of the band, or that it has a helical form. Either explanation would account for its bizarre shapes when the trabecular fibers are viewed in both longitudinal and cross sections.

In histologic preparations, a material which takes an elastic tissue stain surrounds the collagen core.[3] With the electron microscope we have not seen typical elastic fibers adjacent to the central collagen core, but the homogeneous matrix surrounding the core suggested the electron microscopic picture interpreted as elastin (compare background matrix in Figure 6 with "EL" in Figure 5).

Glass membrane

With the electron microscope we have been unable to identify a Descemetlike membrane in the trabeculas similar to that described by Jakus[4] as part of the cornea. We have seen a basement membrane beneath the endothelium varying in thickness from 1,000 Å to 5,000 Å. This membrane is very likely below the resolution of the light microscope, and is probably not the structure described by Salzmann.

Endothelial cells

All trabecular fibers observed thus far have been covered with endothelium. The cells have a discrete cell membrane and a cytoplasm that frequently appeared to extend over the entire trabecular surface. Two adjacent trabecular fibers often shared the cytoplasm of a single cell, and there was an overlapping of the cytoplasm of adjacent endothelial cells.

The cytoplasm contained numerous mitochondria of varying sizes and shapes, and with the usual double inner membranes. Typical melanin pigment granules have also occasionally been seen within the cytoplasm. Whether these represented phagocytosed pigment debris, or were an indication that these cells have a propensity for pigment-formation, is not known.

The nuclei of the cells were round, oval, lobulated, or indented, and in some sections they showed a well-developed nucleolus (fig. 7).

The endothelial cells of the trabeculas failed to show the same excellence of cytoplasmic detail as the nearby muscle cells of the ciliary body. Possibly the cells of the trabeculas, in spite of their accessible position along an "open" channel, are not penetrated by the osmic acid fixative as rapidly as the cells of the ciliary muscle. If there were a film of hyaluronic acid coating the trabeculas, it could account for the apparently poor fixation of the endothelial cells. However, the presence of hyaluronic acid is not ordinarily demonstrable with the electron microscope. On the other hand, this variation in the preservation of cellular detail may simply be a property of the cell-type itself; the endothelial cell may be subject to more rapid postmortem change.

Schlemm's canal

The cells of the endothelium lining the inner wall of Schlemm's canal differed somewhat from the cells covering the trabeculas. The cytoplasm of the canal cells contained one or more giant vacuoles which often gave them a "signet ring" appearance (fig. 8). The vacuolated space within the cells appeared to be devoid of material opaque to

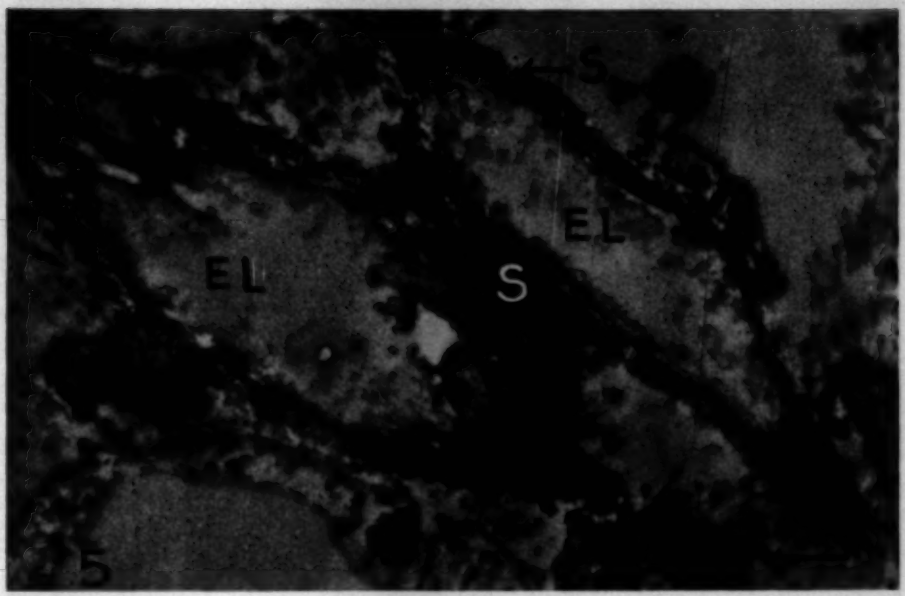

Fig. 5 (Garron, et al.) Elasticlike fibers (EL) cut diagonally showing the covering sheath of fibers with 1,000 Å periodicity (S). (×11,400.)

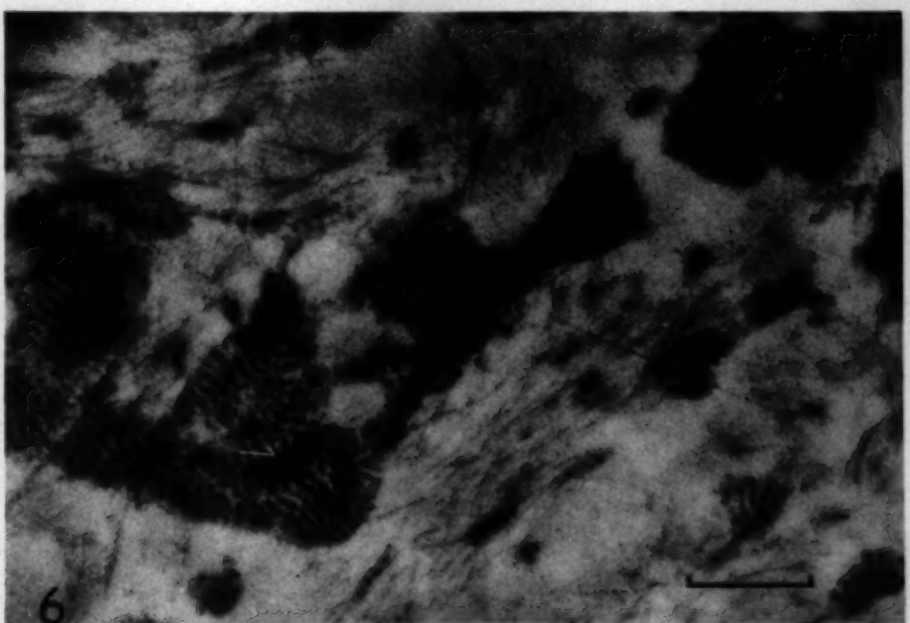

Fig. 6 (Garron, et al.). Electron micrograph showing the components found in the matrix of the trabecular fiber. The large irregular structures have a periodicity of approximately 1,000 Å. Very fine fibrils showing no periodicity are scattered through the matrix. (×20,000.)

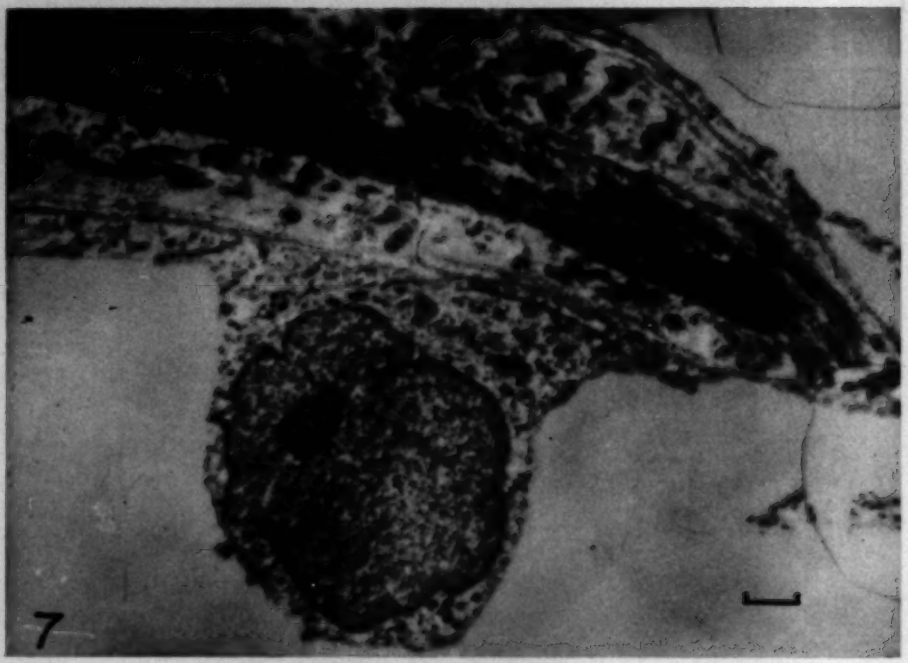

Fig. 7 (Garron, et al.). An oblique section through a trabecular fiber showing an endothelial cell with a lobulated nucleus. Mitochondria may be seen in the cytoplasm.

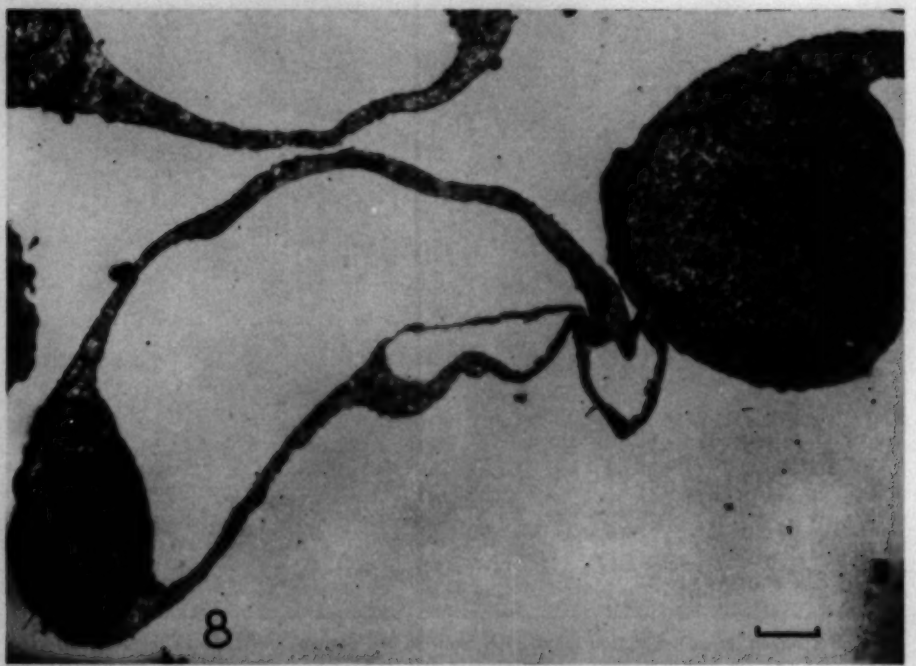

Fig. 8 (Garron, et al.). A single cell from the endothelial lining of the inner wall of Schlemm's canal. The cytoplasm of the cell contains a giant vacuole giving it a "signet ring" appearance, with the nucleus situated eccentrically.

electrons. Serial sections would probably show whether these spaces were "balloon" spaces within the cytoplasmic membrane or toroidal corridors through the cells. The further possibility that they may be artifacts caused by the fixing or embedding processes seems unlikely since the endothelial cells of the trabeculas do not show this type of vacuolization. That they may represent a degenerative change due to age or disease must also be considered.

It should be noted that there did not appear to be a direct opening from Schlemm's canal into the trabecular spaces. Since we have previously demonstrated by other means the openings which complete the pathway of the aqueous humor from the anterior chamber into the canal of Schlemm,[5,6] it was interesting that there has been no evidence of such openings in the electron micrographs taken for the purposes of this study. It could not be regarded as surprising, however, as it can be readily shown by calculation[7] that there is a tissue barrier of low porosity (few holes per unit area) that accounts for the normal resistance to outflow. The chance of finding one of these openings in a thin section is remote.

Summary and Conclusions

1. Examination of the trabecular meshwork with the electron microscope showed the usual structure of an individual corneoscleral trabecular fiber to be as follows: There is a central core of typical collagen fibrils with the usual 640 Å banding. This core is covered by a layer of material with a 1,000 Å periodicity. The core and covering are embedded in an amorphous material of light density containing very fine fibrils and clumps or curls of material with a 1,000 Å periodicity. "Elastic fibers" are distributed irregularly in the outer zone of the matrix. There is a basement membrane beneath the endothelium which covers the trabeculas.

2. The inner wall of Sclemm's canal is lined with endothelial cells, many of which contain giant vacuoles.

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THE DISTRIBUTION OF GLUTATHIONE AND PROTEIN SULFHYDRYL GROUPS IN CALF AND CATTLE LENSES*

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INTRODUCTION

Because of the possible importance of the protein sulfhydryl (PSH) groups in cataract formation[1] and the presence of the high concentrations of glutathione in the lens, the –SH groups of this ocular tissue have been the subject of many investigations.[2-4] Greatly facilitating the study of the –SH groups has been the development of the amperometric titration method. With this convenient and specific procedure for measuring this chemical grouping, it was possible to demonstrate that the reactivity of the –SH groups of the lens proteins is considerably different from those of glutathione.[5] However, no evidence was obtained pertaining to any apparent relationship between glutathione and the protein –SH groups. In the hopes of demonstrating the possible interactions between the protein and non-protein –SH groups, a study is reported here dealing with the distribution of these groups in the lenses of calves and cattle.

METHODS

The cattle lenses (1.8 to 2.3 gm.) and calf lenses (0.8 to 0.95 gm.) obtained from freshly slaughtered animals were decapsulated and the soft outer layers of fibers were teased away from the hard innermost part. The cortex and nucleus separated in this manner from several lenses were pooled and weighed. These fractions were then homogenized in 10 volumes of isotonic Tris buffer pH 7.4 under an atmosphere of nitrogen in a Waring blendor at 5°C. Total –SH groups were measured on this homogenate by the amperometric titration method. Glutathione measurements were made by the standard nitroprusside method[5] on a trichloroacetic acid filtrate of the homogenates.

The amperometric titration method used was essentially that reported by Benesch, et al.[6] The Hg–HgO saturated $Ba(OH)_2$ electrode of Samuelson and Brown[7] was used as the reference electrode. Since this electrode has a potential of −0.10 volts against the saturated calomel electrode no external potential was necessary. The amount of –SH titrated was approximately one μM and the titration was carried out in a Tris buffer solution pH 7.0 made by mixing 4.0 ml. of 1M Tris, 3.4 ml. of 1M nitric acid, 0.3 ml. of 1M potassium nitrate, and enough water to make the total volume 30 ml. The –SH was titrated with 0.001M $AgNO_3$ added in 0.1 ml. increments from a microburette. Nitrogen was passed into the titrating mixture to prevent possible oxidation during the titration. The end-point was determined by plotting the diffusion current, detected by a galvanometer, against the amount of $AgNO_3$ added as described by Benesch, et al.[6]

For the fractionation of the cattle lens proteins, 60 ml. of 6.0-percent water homogenate of lens cortex and 140 ml. of 8.0-percent homogenate of lens nucleus served as the starting material. All fractionation procedures were carried out in a cold room (5°C.). The isolation of the alpha fraction of the cortex and the nucleus was achieved by an isoelectric precipitation at pH 5.0.

The precipitate was recovered by centrifugation and dissolved in a veronal buffer pH 8.6. The alpha fraction so obtained was

* From the Howe Laboratory of Ophthalmology and Department of Biochemistry, Harvard Medical School, and Massachusetts Eye and Ear Infirmary. This work was supported in part by the United States Atomic Energy Commission, contract No. AT (30-1) 1368, and by a graduate training grant P.H.S. No. 2-B-5096 from the National Institute of Neurological Diseases and Blindness, Public Health Service.

subjected to repeated precipitations until it appeared homogenous by paper electrophoresis. Before the beta fraction could be isolated from the supernatant fluid after the isoelectric precipitation, it was necessary to remove completely the unprecipitated alpha proteins. As described by François, et al.,[8] this was accomplished by precipitating the alpha proteins at pH 7.0 with 13-percent alcohol concentration and then followed by another precipitation at 17-percent alcohol.

The beta fraction was then isolated by adding to the supernatant fluid an equal volume of saturated ammonium sulfate solution. The precipitate was dissolved in 10 to 20 ml. of veronal buffer pH 8.6 and made up to a total volume of 50 ml. with water. The beta fraction isolated in this manner from both the nucleus and cortex was reprecipitated by adding enough alkaline saturated ammonium sulfate solution until a concentration of 26 percent with respect to this salt was reached.

The alkaline ammonium sulfate solution was prepared by adding 7.0 ml. of ammonium hydroxide to one liter of saturated ammonium sulfate solution. Several precipitations with alkaline ammonium sulfate (26 percent) were necessary before a homogenous fraction was obtained.

The third fraction, gamma proteins, found only in the cattle lens nucleus was isolated after the alpha and beta fractions were removed. The gamma fraction was precipitated by adding solid ammonium sulfate until 100-percent saturation of this salt was obtained in the supernatant fluid. It was recovered by centrifugation, dissolved in 10 ml. of veronal buffer, and diluted to 50 ml. with water. This fraction was often contaminated with beta proteins which were removed by the addition of alkaline saturated ammonium sulfate solution to 26-percent concentration. The gamma fraction was then precipitated when the alkaline ammonium sulfate was raised to 50-percent concentration.

Estimations of the lens protein fractions were possible from the paper electrophoretic patterns. The various types of lens proteins on the electrophoretogram were made visible by staining with brom-phenol blue. The stained separated fractions were cut out and extracted with constant shaking for 30 minutes in a test tube containing 10 ml. of a mixture of 50-percent methanol and 10-percent Na_2CO_3 solution. The extracted colored solution was then read on a Beckman DU at 595 mμ.

Results and Discussion

In the study on the distribution of protein sulfhydryl (PSH) groups and glutathione (GSH) in the lens it was at once apparent that the concentration of PSH in the nucleus of cattle lens was at a much higher level than that of the cortex. This observation seemed contrary to what was expected, principally because it has been shown that the GSH was concentrated in the lens cortex.[3] Since a close relationship between GSH and PSH has been suspected, it seemed natural to assume that the lens cortex would also contain a high concentration of protein sulfhydryl groups. This assumption proved to be incorrect as pointed out by the results in Table 1. In this study the concentrations of the total sulfhydryl groups and GSH were determined and the PSH content was obtained by the difference of these two values. The concentrations are expressed in micromoles (μM) per gm. dry weight of lens cortex, of lens nucleus, or of whole lens.

The results indicated that the glutathione concentration of the cortex was much higher than that of the nucleus. In the cortex of the cattle lens, the GSH concentration was 53 μM per gm. dry weight of lens cortex while that of the nucleus was 11 μM per gm. dry weight of nucleus.

On the other hand, the concentration of PSH of the nucleus was much higher than that of the cortex. In the nucleus the protein sulfhydryl concentration was observed to be 115 μM/gm. and that of the cortex was 79 μM/gm. The interesting aspect of this

TABLE 1

DISTRIBUTION OF SULFHYDRYL GROUPS IN BOVINE LENS

	Cattle Lens			Calf Lens		
	Cortex	Nucleus	Whole Lens	Cortex	Nucleus	Whole Lens
Total —SH	132.0±3.8	126.0±1.5	130.3±10.0	160.0±17.0	168.1±12.0	163.2±8.0
Glutathione	53.3±9.0	11.4±2.3	26.2± 1.8	43.0± 7.0	16.0± 1.8	29.8±4.0
Protein —SH	79.0±7.1	115.0±1.2	104.1±11.9	117.0±16.0	152.4±11.9	133.4±6.0

The results are given in μM/gm. dry weight as the mean of at least 12 observations ± the standard deviation.

distribution was that the total sulfhydryl content of the nucleus and that of the cortex was almost identical. That is, the sum of the concentrations of GSH and PSH was about equal in the two parts of the lens.

These findings suggest that there may exist some relationship between the nonprotein and the protein sulfhydryl groups in the lens.

It is noteworthy that glutathione occurs in such striking high concentrations in the lens cortex. In the cattle lens where a cleaner separation into the two parts is possible it was shown that the glutathione is five times higher in the cortex than in the nucleus. The GSH concentration of 53 μM/gm. in the lens cortical fibers is much higher than that observed in any other tissues. No definite explanation for this fact can as yet be offered.

There are a number of factors contributing to the concentration of glutathione in the lens cortex. The synthesis of this tripeptide undoubtedly occurs in the cortex. The cortex, including the epithelium, is the site of active metabolism and one mechanism leading to the formation of glutathione is through the action of the enzyme, glutathione reductase.[9] The other factor to consider is that glutathione may be bound in some manner. The evidence for this is that glutathione is difficult to extract from the intact lens.[4] However, a solution containing 3M urea greatly facilitates the extractability of the lens glutathione. Also, there is the observation that when lens is incubated in Tris buffer containing 10^{-3}M cupric ion and equilibrated with 100-percent O_2 only a small amount of the lens glutathione is oxidized.[5]

These findings suggest that glutathione does not appear freely diffusible in the lens, otherwise oxidation of glutathione should have occurred under these conditions. Oxidation of lens glutathione does occur under the same conditions if the lens is homogenized.[5] Apparently the process of homogenization releases glutathione from its binding sites. This does not seem too implausible since glutathione, being a tripeptide, does not have many sites to establish hydrogen bonds.

One possible evidence against the bound state of lens glutathione was indicated when the lens cortical fibers were subjected to prolonged dialysis. The dialysis medium was an isotonic solution buffered at pH 7.4 and included 0.05M EDTA. The dialysis was carried out under nitrogen for 48 hours at 5°C. It was found after dialysis that the concentration of glutathione in the dialysis bag containing the teased cortical fibers was the same as the dialyzing medium. If glutathione were bound it would have been anticipated that the glutathione concentration in the lens cortex would have remained higher than in the dialyzing medium. On the other hand, it could be argued that the conditions used for dialysis were not suitable to maintain the integrity of the lens fibers and thus the glutathione was released from the weak binding sites. It is obvious that further

studies are required before a more satisfactory understanding of the state of glutathione in the lens can be attained.

The unusual differences in the concentrations of glutathione and the protein sulfhydryl groups in the cortex and the nucleus do not appear to depend on the age of the lens. This became apparent when a similar study was made on calf lenses.

In these younger lenses a little more difficulty was encountered in separating the cortex from the nucleus, but, nevertheless, the results confirmed the findings with those observed from the cattle lenses. As in the older lenses the glutathione concentration of the cortex was higher than that of the nucleus, while the protein sulfhydryl concentration was higher in the nucleus.

As indicated in Table 1, the glutathione concentration in the cortex was 43 μM/gm. while it was 16 μM/gm. in the nucleus. On the other hand, the protein sulfhydryl content was observed to be higher in the nucleus, 152 μM/gm., than that of the cortex, 117 μM/gm. Furthermore, as observed in the cattle lens, the total sulfhydryl content of the cortex and the nucleus of the calf lens was approximately the same. In the calf cortex the total sulfhydryl content was 160 μM/gm. and that of the nucleus was at 168 μM/gm.

There appears to be some difference in the total sulfhydryl content of the calf and that of the cattle lenses. As shown in Table 1, the concentration of the total sulfhydryl group in the calf lens was 163 μM/gm. in contrast to a value of 130 μM/gm. for the cattle lens. This difference apparently is not due to the glutathione content, but rather due to the higher protein sulfhydryl content in the calf lens. A possible explanation for this difference was suggested by studying the types and quantities of the lens proteins.

Although these results must be considered as preliminary observations, a separation of the various protein fractions of the cortex and nucleus was accomplished and the sulfhydryl content of each of these fractions

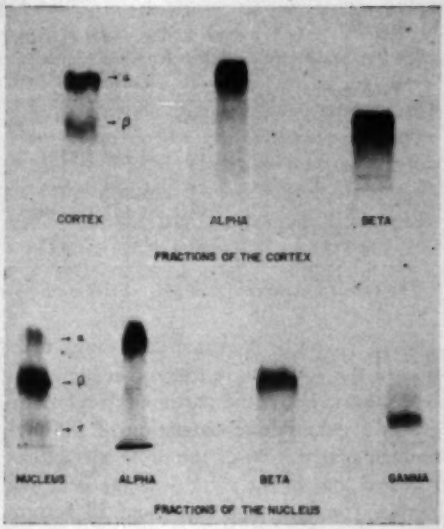

Fig. 1 (Kinoshita and Merola). Electrophoretic patterns for isolated lens proteins.

was determined. These fractions appeared homogenous by paper electrophoresis (fig. 1). However, the purity of these fractions has not as yet been determined by other criteria.

The –SH content for each of the fractions is shown in Table 2. The actual values must be viewed with some caution since oxidation of some of the –SH groups may have occurred during the isolation procedures and the exact purity is not known. However, these results serve to give some idea as to the –SH content of the three major types of cattle lens proteins. Under the conditions by which these fractions were obtained, it appears that the alpha fraction is a class of protein which is "poor" in –SH groups while the beta and gamma fractions may be regarded as –SH rich proteins.

From the electrophoretic patterns shown on Figure 2, assuming that the –SH contents of the various proteins of calf and cattle lenses are approximately the same, it appears that the presence of greater concentrations of beta and gamma proteins in the calf lens accounts for its higher –SH

TABLE 2

THE —SH CONTENT OF THE LENS PROTEINS

Preparation Number	Cortex		Nucleus		
	Alpha	Beta	Alpha	Beta	Gamma
1	70	167	57	164	132
2	70	139	37	131	169
3	75	162	80	154	126
4	61	150	40	170	115
Average:	69	155	53	155	136

The values are expressed in μM —SH/gm. dry weight.

content. It can be seen that the gamma fraction in the calf lens is present in high concentrations. From the paper electrophoretograms a quantitative estimation of the percentage composition of the protein fractions revealed that in the cattle lens the beta and gamma fractions make up about 55 percent of the total protein. In contrast to this, a much higher amount, 82 percent, of these –SH rich proteins are found in the calf lens.

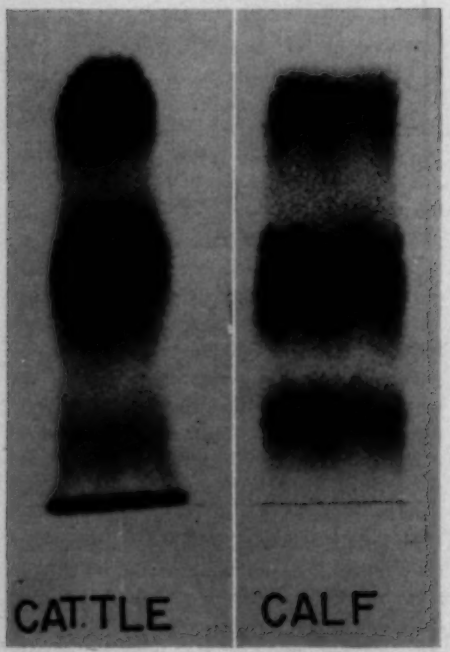

Fig. 2 (Kinoshita and Merola). Electrophoretic patterns of lens proteins of calf and cattle.

In this estimation the insoluble fraction, the albuminoid, is also taken into consideration. The albuminoid fraction is found in higher concentration in the older lens (15 percent) than in the young lens (5 percent). Therefore, the greater proportion of beta and gamma proteins in the calf lens probably accounts for the higher –SH content in the proteins of the younger lens.

The same basis may be used to explain the higher –SH content in the lens nucleus than that found in the cortex, as shown by Krause,[10] who studied the protein composition of the lens from the outermost fibers to the central nucleus and found that the concentration of the beta fraction is the same in the cortex as in the nucleus. However, in the nucleus he found that there is an additional class of proteins, the gamma fraction. François, et al.[8] also found that the sum of the beta and gamma proteins is higher in the nucleus than in the cortex. In view of the demonstration that the gamma proteins are rich in –SH groups, its presence in the nucleus would contribute to a higher –SH content.

It is intriguing to speculate the possible significance of the distribution of the glutathione and protein sulfhydryl groups in the cortex and nucleus. The distribution of these –SH groups for cattle and calf lenses is summarized in Figure 3.

Since a relationship between the nonprotein and protein –SH groups is suggested by

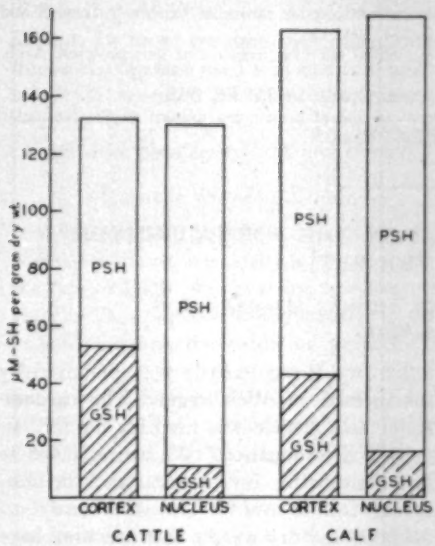

Fig. 3 (Kinoshita and Merola). Distribution of sulfhydryl groups in calf and cattle lenses.

this study, it makes one wonder whether it is correlated to the ageing process of the lens fibers. In the development of the lens it appears that as new fibers are elaborated at the equator of the lens from the epithelial cells, the oldest fibers of the cortex are gradually compressed into the nucleus. As this occurs, it is suggested from the results obtained here that changes in the nonprotein and protein sulfhydryl concentrations also take place. That is, as the proteins of the cortex are converted to that of the nucleus there occurs a drop in glutathione content and an increase in protein sulfhydryl groups. It appears that the –SH group of glutathione is in some way converted to a protein –SH.

One possible mechanism to explain this observation is that glutathione may participate in a disulfide exchange reaction. In this case the glutathione sulfhydryl group may react with the protein disulfide linkage liberating a protein sulfhydryl group and forming a glutathione-protein mixed disulfide linkage. Whether this mechanism or one similar to it takes place and what effect it may have in determining the state of lens protein are a matter of future investigations.

Conclusion

A study of the distribution of the glutathione and protein sulfhydryl groups in the cortex and nucleus of lenses from calves and cattle was undertaken. In both the young and older lenses the cortex had a greater concentration of glutathione than the nucleus. On the other hand, the nucleus possessed the higher concentration of protein –SH groups. The sum of the concentrations of glutathione and protein sulfhydryl groups was equal in the two parts of the lens.

Isolation of the three major types of lens proteins suggested that the beta and gamma proteins are –SH rich, while the alpha proteins have a low –SH content. The higher proportion of beta and gamma proteins in the younger lens accounts for the greater –SH content than that observed for the older lens.

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THE EFFECT OF GLUTATHIONE ON THE FORMALDEHYDE OXIDATION IN THE RETINA*

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INTRODUCTION

In view of the investigations by Potts and Johnson[1] suggesting that formaldehyde is the proximal toxic agent in methanol poisoning, it appeared important to investigate the mechanisms by which formaldehyde is metabolized in the retina. Previously it was generally believed that formaldehyde oxidation by animal tissues is catalyzed by a non-specific enzyme, the acetaldehyde dehydrogenase. In this process formaldehyde, like other aldehyde compounds, is oxidized to its corresponding acid and diphosphopyridine nucleotide (DPN) is the compound reduced. However, Strittmatter and Ball[2] demonstrated that in liver there is a specific enzyme which oxidizes formaldehyde. The interesting feature of this enzyme, formaldehyde dehydrogenase, is that it requires glutathione as a cofactor. This report presents evidence which suggests the occurrence of this specific enzyme in cattle, rabbit, and human retinas.

METHODS

All measurements for DPN reduction were followed at room temperature at 340 mμ with a Beckman model DU spectrophotometer. Acetaldehyde was purified by distillation and formaldehyde was prepared by the method of Weinberger.[3] The chromotropic acid method was used for the formaldehyde determination.[4] We are indebted to Dr. Strittmatter for his assistance in measuring formic acid.[4,5] The other analytical methods and dry weight determination have been previously described.[6]

In the experiment with cattle retina the tissue preparations were as follows: retinas were homogenized in three volumes of 0.1M phosphate buffer pH 7.4, centrifuged at 15,000 × g, and the residue discarded. The supernatant fluid was then treated with a final concentration of 70 percent ammonium sulfate. The resulting percipitate recovered after centrifugation was dissolved in phosphate buffer and then precipitated again with 70 percent ammonium sulfate. The precipitate was dissolved in one-third the original volume of isotonic saline. Any insoluble material was removed by centrifugation. The retina extract prepared in this manner was free of endogenous substrate and DPN. This preparation is referred to as the cattle retinal extract in the text. Rabbit and human retinal extracts were prepared by homogenizing in equal volumes of phosphate buffer and then dialyzed against 100 volumes of water.

The description of the reaction mixture to measure DPN reduction was as follows: 20 μM of formaldehyde or acetaldehyde, 0.2 μM of DPN, and 2.0 to 3.0 mg. dry weight of the retinal extracts and enough 0.1M

* From the Howe Laboratory of Ophthalmology and Department of Biochemistry, Harvard Medical School, and Massachusetts Eye and Ear Infirmary. This work was supported in part by the United States Atomic Energy Commission, contract No. AT (30-1)-1368.

Tris buffer pH 8.0 to make a final volume of 3.0 ml. In those cuvettes containing glutathione the amount used was one mg. unless otherwise specified. For the experiment to establish the pH optimum the buffers employed were phosphate, Tris, and Borate.

Results and Discussion

It is convenient to follow the oxidation of formaldehyde or acetaldehyde by measuring the rate of DPN reduction spectrophotometrically. This experimental approach is permissible because the oxidation proceeds by the following equation for formaldehyde oxidation: $CH_2O + DPN = CHOOH + DPNH$, or for acetaldehyde oxidation: $CH_3CH_2O + DPN = CH_3COOH + DPNH$.

The oxidation of formaldehyde and acetaldehyde by a preparation of cattle retinal extracts is shown in Figure 1. As measured by the rate of DPN reduction, the acetaldehyde oxidation appears to proceed at a much faster rate than the formaldehyde oxidation. When neither acetaldehyde nor formaldehyde is present there is very little reduction of DPN as indicated by the curve 0. There is a striking acceleration of DPN reduction when glutathione is added to the reaction mixture with formaldehyde as the substrate. In contrast to this, the addition of glutathione exerts no effect on the DPN reduction when acetaldehyde is the substrate. Glutathione can not be substituted by cysteine, cysteinyl glycine, or oxidized glutathione. It, therefore, appears that glutathione is specifically required in this reaction and other sulfhydryl containing compounds or the oxidized form of glutathione are unable to replace it. These results indicate that since formaldehyde oxidation is dependent on the presence of glutathione, the formaldehyde dehydrogenase appears to be functioning in the retina.

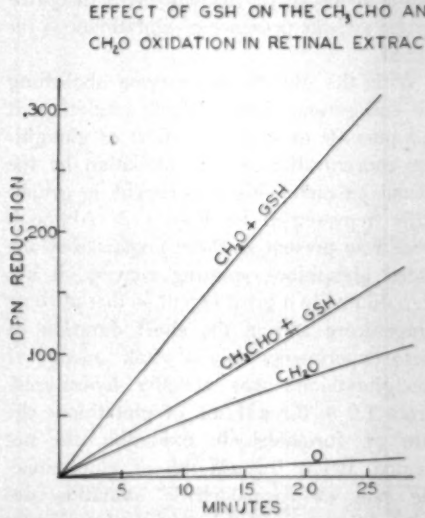

Fig. 1 (Kinoshita and Masurat). The effect of glutathione on acetaldehyde and formaldehyde oxidation in cattle retinal extract.

A quantitative estimation of formic acid was undertaken to demonstrate that this acid is the product of formaldehyde oxidation. In this experiment with formaldehyde as the substrate, DPN was added to the retinal extracts. Since the stoichiometry of this reaction indicated that for every molecule of formaldehyde oxidized a DPN molecule is reduced, considerable amounts of DPN would be needed to yield a measurable amount of formic acid. To circumvent this problem, sodium pyruvate and yeast crystalline lactic acid dehydrogenase (Worthington) were also added to the reaction mixture. Pyruvate, catalyzed by lactic acid dehydrogenase, served to reoxidize the DPNH formed in the oxidation of formaldehyde. Since under these experimental conditions the oxidation of formaldehyde was coupled with pyruvate reduction, only a small addition of DPN was required. The results of this experiment are shown in Table 1. Without the presence of glutathione only small amounts of formaldehyde were oxidized or formic acid produced. However, when glutathione was added to the reaction mixture considerably more formaldehyde was oxidized and formate produced. These results suggested that for every micromole of formaldehyde oxidized there was a micromole of

TABLE 1

FORMALDEHYDE OXIDATION IN CATTLE RETINAL EXTRACT

Reactants Utilized	Glutathione		Products Formed	Glutathione	
	−	+		−	+
Formaldehyde	0.8	2.3	Formic Acid	0.5	2.7
Pyruvate	0.5	3.0	Lactic Acid	0.4	3.4

20 μM of formaldehyde and pyruvate, 0.2 μM of DPN were incubated with cattle retinal extract (15 mg. dry wt.) and 1 mg. of yeast lactic acid dehydrogenase in 3 ml. of 9.1 M phosphate buffer pH 7.5. In those tubes containing glutathione, the amount was 3.0 μM. The reaction was allowed to proceed for one hour at 37°C.

formic acid produced. Indicative of the coupling of the formaldehyde oxidation reaction with pyruvate reduction was that a similar stoichiometry was observed between the disappearance of pyruvate and the production of lactate.

In these preparations of the retinal extracts there occurred a measurable amount of formaldehyde oxidation even without the addition of glutathione as evidenced by the results in Figure 1 and Table 1. It seemed worth while to establish whether this oxidation was due to small traces of glutathione present in these preparations or if the oxidation was catalyzed by the acetaldehyde dehydrogenase of the retina. One approach to this problem was to remove any trace of glutathione from this preparation by treatment with glutathionase, an enzyme which hydrolyzes this tripeptide. This was accomplished by incubating the retinal extracts with glutathionase purified with kidney[7] for 30 minutes at 37°C. When the retinal extract was treated with the glutathione hydrolyzing enzyme, it lost the ability to oxidize formaldehyde. The addition of excess glutathione to the incubating medium restored the ability of the extract to oxidize formaldehyde. These results suggested that in the retina, formaldehyde oxidation proceeds primarily by the action of the formaldehyde dehydrogenase and the presence of glutathione is required.

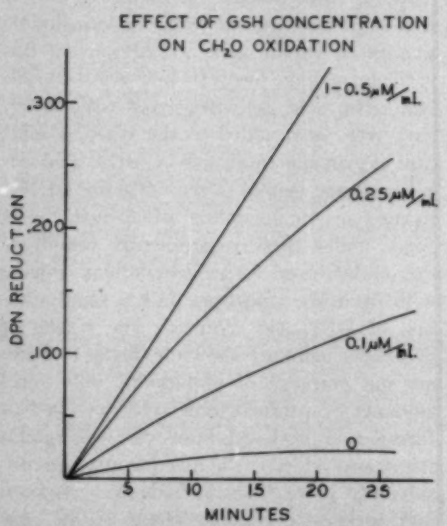

Fig. 2 (Kinoshita and Masurat). The effect of glutathione concentration on the formaldehyde oxidation in cattle retinal extract.

With the glutathione enzyme abolishing the endogenous formaldehyde oxidation, it was possible to study the effect of glutathione concentration on the oxidation by the retinal extract. This experiment is graphically represented in Figure 2. Although there was present in these preparations the added glutathione splitting enzyme, it has been diluted to a great extent so that at room temperature and in the short duration of these experiments only a small amount of the glutathione was actually hydrolyzed. From 1.0 to 0.5 μM/ml. of glutathione the rate of formaldehyde oxidation did not change. Below 0.5 μM/ml. of glutathione, the rate of formaldehyde oxidation decreased, but even at 0.1 μM/ml. the stimulatory effect of glutathione was easily apparent.

The results seem to suggest that only catalytic amounts of glutathione are necessary for the formaldehyde oxidation in the retina. The role of glutathione in formaldehyde oxidation is not known. It is thought that before the oxidation occurs, glutathione combines through its sulfhydryl group with formaldehyde. This transient intermediate is then attacked by the enzyme. Glutathione serving in this capacity functions similarly in the oxidation of another aldehydic metabolite, glyceraldehyde phosphate.[8]

It was possible to demonstrate the presence of the formaldehyde dehydrogenase in the rabbit and human retinas. In Figure 3 is shown the course of formaldehyde oxidation in a dialyzed preparation of human retinal extracts. As shown in Figure 3, when formaldehyde, dialyzed retinal extract, and DPN were incubated no reduction of DPN was observed. After five minutes, the addition of glutathione initiated the reduction of DPN. The reduction of DPN began slowly at first and then proceeded at a uniform rate. No definite explanation is available to account for the latent period observed. It may

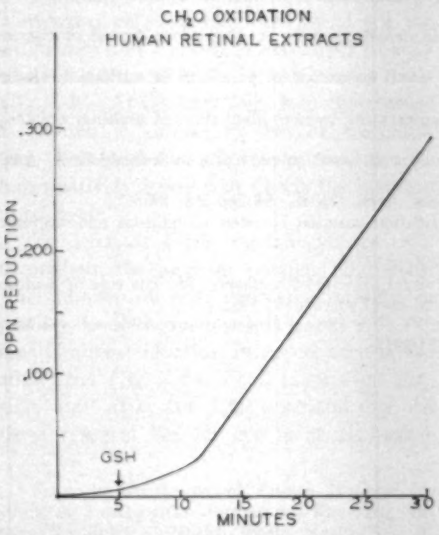

Fig. 3 (Kinoshita and Masurat). Formaldehyde oxidation in human retinal extracts.

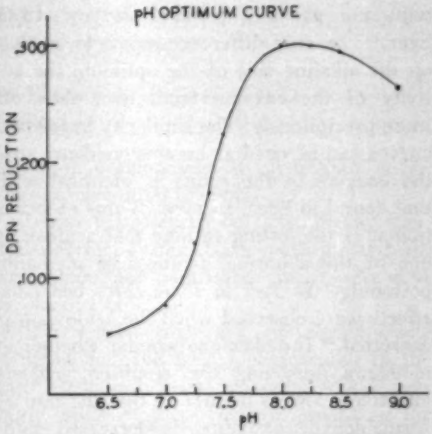

Fig. 4 (Kinoshita and Masurat). pH optimum curve for cattle retinal formaldehyde dehydrogenase.

be possible that the latent period is a reflection of the interaction between glutathione and formaldehyde as suggested by Strittmatter and Ball.[2]

One property of the formaldehyde dehydrogenase of especial interest is the pH optimum curve. The enzyme activities were determined by incubating 3.0 mg. of cattle retinal extract, 0.2 μM DPN, and 1.0 mg. of glutathione and measuring the amount of DPN reduced 10 minutes after the addition of 20 μM of formaldehyde. As indicated in Figure 4 the pH optimum for the formaldehyde oxidation by the cattle retinal extracts is at 8.0. On the alkaline side of this pH the enzyme activity decreased rather slowly. In contrast to this, the enzyme activity on the acid side of the optimum drops off very sharply. The pH optimum curve of the formaldehyde dehydrogenase from human retinal extracts is similar but sufficient number of experiments were not done to warrant its presentation here. It appears from the pH optimum curve that a shift of 0.25 pH units from 7.5 to 7.25 results in a drop of about 25 percent in enzyme activity. The pH optimum curve of the cattle retinal extracts is almost identical to that observed

with the partially purified enzyme from liver.[2] The only difference seems to be that on the alkaline side of the optimum the activity of the enzyme from liver falls off more precipitously. The similarity in the two curves can be cited as another evidence that the enzyme in the retina is identical with that found in liver. In view of this observation it is interesting to note that acidosis is one of the abnormal features of methanol poisoning.[9] In fact in some cases beneficial effects were observed when the acidosis was corrected.[10] It makes one wonder whether in methanol poisoning the resulting acidosis effectively lowers the pH so that the rate of formaldehyde oxidation is decreased sufficiently to allow the toxic agent to accumulate to a undesirable level.

From the present investigations it appears that a specific enzyme, formaldehyde dehydrogenase, is found in the retina. As suggested by the investigations of Potts and Johnson,[1] the accumulation of formaldehyde may be important in determining the course of methanol poisoning. The possibility that some change in the activity of the formaldehyde dehydrogenase is of some significance may be worthy of consideration. As yet no definite evidence on the role of this enzyme in methanol blindness has been established. Whether a deficiency of this enzyme or the unavailability of glutathione in the retina contributes to the accumulation of formaldehyde in methanol poisoning requires further study.

Conclusion

In retinal extracts the oxidation of formaldehyde depends on the presence of glutathione. The product of the formaldehyde oxidation is formic acid. The enzyme which catalyzes this reaction appears to be formaldehyde dehydrogenase and has been demonstrated to occur in cattle, human, and rabbit retinas.

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QUANTITATIVE HISTOCHEMICAL CHANGES IN THE DEVELOPMENT OF THE RAT LENS AND CORNEA*

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The development of the eye has been studied extensively from the aspect of its morphology and physiology, but very little from the point of view of its biochemistry. Bembridge has pointed out the fall of vitreous protein and rise of hyaluronic acid that occurs during the first three weeks of life in rabbits.[1] Smelser has studied S^{35} incorporation during corneal development.[2] Enzymatic assays of brain tissue as it undergoes differentiation have been reported previously.[3-6] They indicate remarkable changes with differentiation of this tissue. As a result it was decided to determine quantitatively several enzyme systems in lens and cornea at four stages of development.

The rat was selected for this study because its eye is quite immature at birth. This is illustrated in Figures 1 and 2. At birth (fig. 1-a) the corneal epithelium consists of a single layer of cells and the stroma contains many cells. At the 10th day of age the epithelium now has one to two layers of cells while the stroma still contains many cells (fig. 1-b). At 20 days (fig. 1-c) the corneal epithelium is similar to that of the adult (fig. 1-d) and the stroma appears to contain relatively fewer cells due to the increase in collagen.

The lens, at birth, contains many cells throughout the anterior portion (fig. 2-a). The number of cells decreases steadily so that by the 20th postnatal day (fig. 2-c) the lens is almost identical in appearance to the adult lens (fig. 2-d). Fetal circulation persists until after the 10th postnatal day. In these respects the rat eye is at an earlier stage of development at birth than is the case in most other animals.

Sugita has pointed out that the rat central nervous system lacks maturity at birth and undergoes maturation within the first weeks of life.[7,8]

The major pathways of carbohydrate metabolism have been demonstrated in the eye.[9] Wortman[10] has recently found very high levels of lactic dehydrogenase in the corneal epithelium of the rabbit. He has also identified enzymes of the citric acid cycle and hexose monophosphate shunt in homogenates of whole lens and lens capsule.[11]

In this work various parts of lens and cornea obtained from animals 0, 10, 20, and 100 days of age were assayed for the following enzymes: hexokinase, aldolase, and lactic, isocitric, glucose-6-phosphate, and glutamic dehydrogenases.

MATERIALS AND METHODS

Eyes from newborn, 10-, 20-day, and adult (100 day) albino rats of the Sprague Dawley strain were enucleated and rapidly frozen in liquid nitrogen. Two litters provided all the animals used in this work. Three animals were used at each age level. Frozen sections, 15 microns thick, were cut. These were lyophilized at −20°C. as described by Lowry.[12]

The frozen-dried material was dissected and appropriate areas selected for analysis. Samples ranging from 0.3 to 5.0 micrograms were weighed to an accuracy of one percent with the Lowry quartz fiber balance,[13,14] placed in micro test tubes two mm. in diameter, and stored at −30°C. for short periods before analysis.

The lens of newborn animals was divided into six or seven segments about its center. At other ages it was possible to obtain sam-

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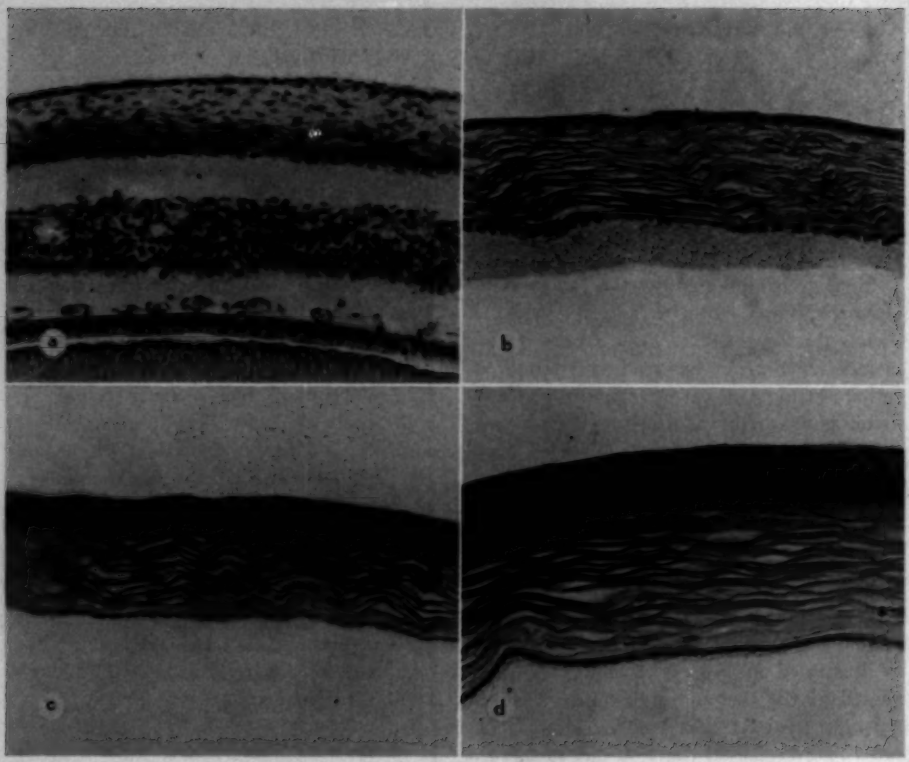

Fig. 1 (Kuhlman and Resnik). Histologic changes accompanying postnatal corneal development of the rat. (×430.)
a. Newborn cornea with iris and lens capsule.
b. Cornea of rat 10 days of age.
c. Cornea from rat 20 days of age.
d. Cornea from adult rat.

ples of cortex and nucleus. It was not possible to obtain lens capsule from newborn and 10-day-old animals. Lens capsule from older animals was found to contain epithelium when studied histologically. Newborn corneal epithelium always contained an edge of stroma.

LDH,* G6DH and GDH were assayed by the methods of Robins[15] and Lowry, *et al.*[16–20] HK was assayed by the oxidation of glucose-6-phosphate with a purified preparation of Zwischenferment.† The reduction of TPN^+ was followed by measuring the TPNH formed fluorometrically. Standards showed a linear relationship between the conversion of glucose-6-phosphate and the reduction of TPN^+. The method of Lowry[21] was used for the assay of ICDH. Conditions are adjusted to be optimum for each system. Tissue sections are diluted more than 1,000

* The following abbreviations will be used: LDH, lactic dehydrogenase; G6DH, glucose-6-phosphate dehydrogenase; GDH, glutamic dehydrogenase; ICDH, isocitric dehydrogenase; and HK, hexokinase. In addition, TPN^+ and TPNH represent the oxidized and reduced forms of triphosphopyridine nucleotide. MKH represents moles of substrate oxidized, or split, per kilogram dry weight of tissue per hour.

† The authors are indebted to Dr. O. H. Lowry for making this method available to us for use at this time.

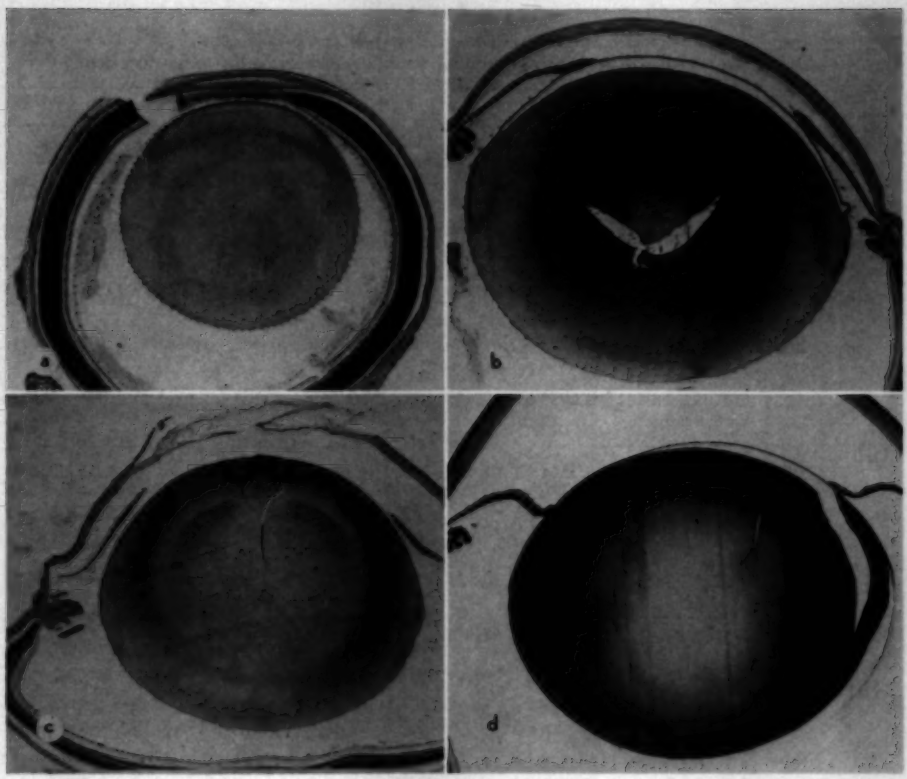

Fig. 2 (Kuhlman and Resnik). Histologic changes accompanying postnatal lens development of the rat. (×40.)
a. Newborn lens (note hyaloid circulation).
b. Lens of 10-day-old rat eye.
c. Lens of 20-day-old rat eye.
d. Lens of adult rat eye.

fold to obviate the influence of any inhibitory substance in the samples themselves.

Results and Discussion

The data obtained are summarized in Table 1 and Figures 3, 4, and 5. In general the changes in activity appear to parallel the changes in histology (figs. 1 and 2). Initially, there is little difference between the enzymatic activity of corneal epithelium and stroma. Enzymatic activity of the corneal epithelium increases until the 20th day when a plateau is reached. The extent to which this can be correlated with increased mitotic activity in the corneal epithelium is not known. It might be of interest to note at this point, however, that the eyelids of the young rat do not open until sometime after the 15th day. In corneal stroma, on the other hand, the activity of all the enzymes measured decreases with age. By the 10th day corneal stroma and epithelium are enzymatically very different structures. The general activity in lens falls concomitantly with the decrease in its cellular content. The epithelial capsule area contains higher activity relative to the cortex.

With advancing age, decrease of enzy-

TABLE 1

ENZYMATIC ACTIVITY AT LOCATION STATED AT VARIOUS AGES

(Activities expressed as moles substrate oxidized or split per kilogram dry weight per hour.)

System	Age	Cornea		Lens		
		Epithelium	Stroma	Capsule	Cortex	Nucleus
		MKH s.e.m.	MKH s.e.m.	MKH s.e.m.	MKH s.e.m.	MKH s.e.m.
G6DH	NB	0.890 ±0.0630 n=4	0.631 ±0.151 n=5	—	—	0.425 ±0.0537 n=5
	10D	1.26 ±0.0457 n=4	0.195 ±0.0519 n=5	—	0.309 ±0.0210 n=5	0.208 ±0.0284 n=5
	20D	2.53 ±0.201 n=3	0.178 ±0.0130 n=5	0.978 ±0.300 n=3	0.402 ±0.0541 n=5	0.130 ±0.0212 n=4
	Ad.	2.08 ±0.0482 n=6	0.058 ±0.00713 n=5	0.518 ±0.0695 n=5	0.163 ±0.0177 n=5	0.096 ±0.0234 n=5
LDH	NB	13.1 ±2.28 n=5	15.4 ±1.61 n=5	—	—	1.38 ±0.206 n=11
	10D	21.3 ±1.17 n=5	10.5 ±0.196 n=3	—	4.80 ±0.324 n=5	3.06 ±0.466 n=5
	20D	27.3 ±2.53 n=8	6.22 ±0.708 n=4	10.5 ±1.81 n=5	3.35 ±0.318 n=5	2.89 ±0.318 n=5
	Ad.	28.6 ±3.24 n=10	2.07 ±0.0410 n=5	10.6 ±1.16 n=5	6.20 ±0.756 n=5	2.13 ±0.270 n=6
ICDH	NB	0.572 ±0.0787 n=5	1.21 ±0.419 n=4	—	—	0.465 ±0.128 n=5
	10D	2.48 ±0.446 n=5	0.476 ±0.147 n=4	—	0.619 ±0.126 n=5	0.476 ±0.0497 n=5
	20D	4.49 ±0.317 n=8	0.0593 ±0.0791 n=3	0.232 ±0.0488 n=5	0.241 ±0.0736 n=5	0.419 ±0.157 n=4
	Ad.	5.41 ±0.506 n=8	0.117 ±0.0258 n=4	0.428 ±0.0805 n=9	0.169 ±0.0603 n=5	0.0933 ±0.0237 n=5
HK	NB	0.252 ±0.0164 n=5	0.257 ±0.0242 n=5	—	—	0.204 ±0.0894 n=5
	10D	0.176 ±0.0158 n=5	0.220 ±0.0440 n=5	—	0.430 ±0.0632 n=3	0.127 — n=1
	20D	0.182 ±0.0595 n=4	0.202 ±0.0356 n=5	0.185 ±0.145 n=4	<0.1	<0.1
	Ad.	0.119 ±0.0187 n=5	<0.1	<0.1	<0.1	<0.1
Aldolase	NB	0.289 ±0.0183 n=3	0.161 ±0.0313 n=4	—	—	0.241 ±0.0292 n=4
	10D	0.295 ±0.0665 n=4	0.195 ±0.0760 n=4	—	0.179 ±0.0585 n=4	0.128 ±0.0619 n=4
	20D	0.219 ±0.0880 n=8	0.137 ±0.0295 n=4	0.530 ±0.0474 n=4	0.196 ±0.0206 n=4	0.164 ±0.0750 n=5

TABLE 1—*Continued*

System	Age	Cornea		Lens		
		Epithelium	Stroma	Capsule	Cortex	Nucleus
		MKH s.e.m.	MKH s.e.m.	MKH s.e.m.	MKH s.e.m.	MKH s.e.m.
	Ad.	0.358 ± 0.0560 n = 3	0.0464 ± 0.0202 n = 5	0.206 ± 0.0585 n = 4	0.189 ± 0.0740 n = 4	0.022 ± 0.0885 n = 4
GDH						
	NB	0.328 ± 0.0846 n = 4	0.164 ± 0.0850 n = 5	—	—	0.190 ± 0.0740 n = 6
	10D	0.207 ± 0.0338 n = 5	<0.1	—	<0.1	<0.1
	20D	0.287 ± 0.0790 n = 6	<0.1	0.254 ± 0.0584 n = 5	<0.1	<0.1
	Ad.	0.141 ± 0.0184 n = 5	<0.1	0.257 ± 0.0783 n = 4	<0.1	<0.1

matic activity is noted in both the lens cortex and nucleus. The activity of the nucleus is seen to be quite low at all ages, and the adult in most cases has the lowest level.

Lactic dehydrogenase

LDH has the highest activity of any enzyme measured. In this study the reaction was measured in the direction of pyruvate formation. When measured in the reverse direction (that is, lactate formation), an activity of 77 MKH was obtained in adult corneal epithelium. Because of the magnitude of this figure, LDH formation was measured in corneal epithelium of rabbit in the direction of lactate formation. The activity in this instance was in excess of 1,500 MKH. This enzyme will be studied in more detail to determine more precisely its activity in cornea. In addition, it would be of interest to know the relationship of this enzyme to the metabolism and function of the cornea. Cat corneal epithelium was found to have an activity of 103 MKH. The general pattern of enzymatic activity during maturation is seen in Figure 3.

deRoetth[22] has demonstrated the cornea's capacity for anaerobic glycolysis. He further noted that the QO_2 of corneal epithelium decreased after it was scraped off and homogenized in buffer. Herrmann and Hickman reported that the lactic acid content of corneal tissue was 100 mg./100 gm. of tissue in the epithelium and 65 mg./100 gm. of tissue in stroma.[23] On this basis, they sug-

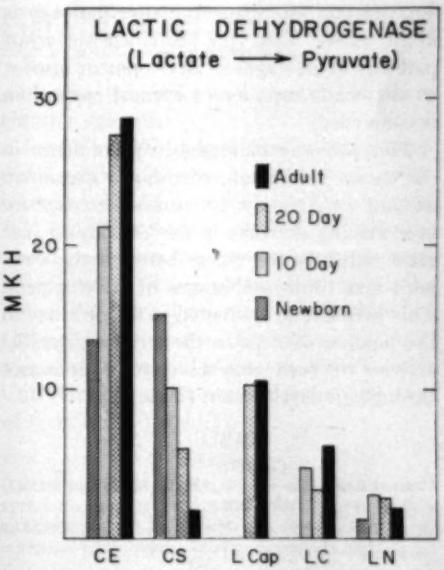

Fig. 3 (Kuhlman and Resnik). Activity of LDH at the indicated age and location expressed as moles of substrate oxidized per kilogram dry weight of tissue per hour. (CE) Corneal epithelium. (CS) corneal stroma. (L Cap) Lens capsule and epithelium. (LC) Lens cortex. (LN) Lens nucleus.

gested that lactic acid is transported against a gradient from stroma to epithelium. From the localization of LDH found in this present study it does not seem that this is necessarily the case. In fact, if one has to predict, it seems that just the opposite would be the case.

Glucose-6-phosphate dehydrogenase and aldolase

The presence of the oxidative pathway has been established by Kinoshita.[24,25] In view of this evidence it may be possible to regard G6DH activity as an indication of the capacity for the oxidative pathway to function (fig. 4). In addition, if the assumption is made that aldolase is an index of the extent to which the Embden-Meyerhof pathway might function, the ratios of these two systems may be taken as an indication of the relative activity of these two pathways. These ratios are presented in Table 2. It appears that the direct oxidative pathway is more active than the Embden-Meyerhof pathway at all stages of development insofar as enzymatic capacity of corneal epithelium is concerned.

This pathway increases in prominence in the cornal epithelium, reaching a maximum around the 20th day. In corneal stroma there is a striking decrease in the G6DH/aldolase ratio noted in the tissue between the newborn and 10-day-old stages of development. This may not be attributed to the decrease in the number of cells in the stroma since the activity of both enzyme systems decreases throughout development (table 1).

TABLE 2

Comparison of $\frac{\text{G6DH}}{\text{Aldolase}}$ ratios in various locations of rat lens and cornea at the ages stated

Age	C.E.	C.S.	L. Caps.	L.C.	L.N.
NB	3.08	3.91	—	—	1.77
10D	4.26	1.00	—	1.72	1.62
20D	11.5	1.30	1.85	2.05	0.793
Ad.	5.81	1.25	2.52	0.863	0.436

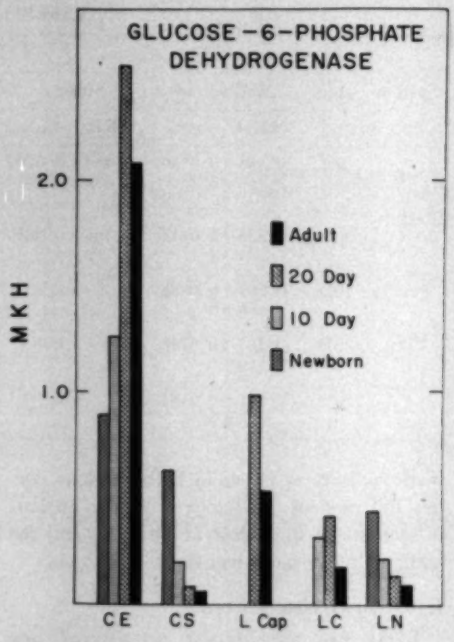

Fig. 4 (Kuhlman and Resnik). Activity of G6DH at the indicated age and location. Abbreviations as in Figure 3.

In the lens cortex and nucleus the relative importance of the oxidative pathway decreases throughout development. In lens capsule-epithelium, however, there appears to be no change in this pathway between 20 days and 100 days of age.

These results, with some qualification, are in agreement with the conclusions of Kinoshita[24,25] concerning the general importance of the oxidative pathway in the metabolism of lens and cornea.

Isocitric dehydrogenase

Kinoshita has suggested that the direct oxidative pathway may play a more conspicuous role than the citric acid cycle.[24] While it is difficult to relate capacity to function, the ICDH levels obtained in this work raise the question, at least in the minds of the authors, that the Krebs cycle may also be important in lens and corneal metabolism.

ICDH levels were found to be greater than those of G6DH in rat corneal epithelium (compare figs. 4 and 5). In the light of our current findings this point appears to warrant further consideration.

Hexokinase, aldolase, and glutamic dehydrogenase

Both hexokinase and aldolase have low activities, generally far below ICDH, G6DH, and LDH (table 1). Levels of HK in lens cortex, nucleus, and corneal stroma are too low to be detected by the methods used in this work. Green, et al.[26] have shown hexokinase to be a limiting step in lens and corneal metabolism. They demonstrated that lactic acid production can be increased by fortifying the incubation media with pure hexokinase. This point is confirmed here and, in addition, a second "narrow" pathway, aldolase, is indicated.

As low as these values for HK are, they agree with the results obtained in studies of glucose consumption of lens; that is, the lens uses 0.14 mg. glucose/mg./hour.[9] The low levels of HK, aldolase, and GDH make it difficult to discern any relationship between growth and the activity of these systems in the various tissues studied; however, they tend to remain unchanged. GDH activity is so low that it probably has little or no importance in the metabolism of lens and cornea. Wortman[10] has been unable to detect glutamic-aspartic transaminase in either lens or cornea.

The activities listed indicate the maximal capacity of a given area of tissue to catalyze a specific reaction. As a result, these may indicate the effective enzyme concentration. In vivo the extent to which a given system is functionally operative is usually less than the absolute potential of a tissue. However, in brain at least, certain steps do not seem to be greatly in excess of metabolic requirements. Among these are hexokinase, aldolase, triosephosphate dehydrogenase, G6DH, ICDH, and others.[27] In this work an effort was made to investigate certain of these limiting systems.

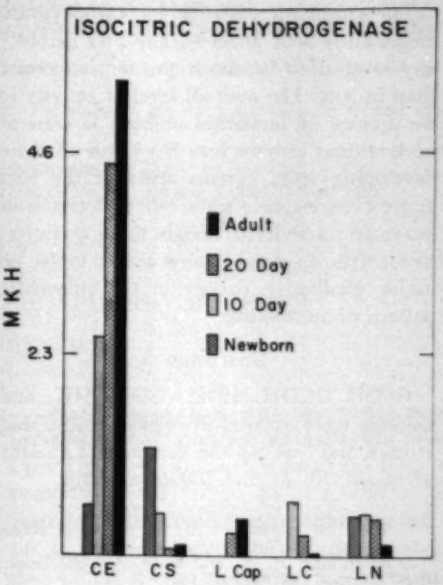

Fig. 5 (Kuhlman and Resnik). Activity of ICDH at the indicated age and location. See Figure 3 for explanation of abbreviations.

Levels of HK and aldolase in corneal epithelium fail to increase from newborn levels, whereas other systems undergo two- to ninefold increases. As a result, HK and aldolase would seem to be proportionately more prominent in tissue obtained at an earlier rather than at a later stage of development (table 3). Hexokinase becomes more limiting with age and aldolase less significant as G6DH rises to enhance the shunt pathways of metabolism (table 2).

TABLE 3

Ratios of G6DH, LDH, ICDH and aldolase activity to HK in developing corneal epithelium

	G6DH / HK	LDH / HK	ICDH / HK	Aldolase / HK
NB	3.53	51.9	2.27	1.15
10D	7.16	121.0	14.1	1.67
20D	13.9	150.0	24.7	1.20
Ad.	17.5	240.0	45.5	3.01

The enzymatic capacity for carbohydrate metabolism both anaerobically and aerobically is seen to be much greater in cornea than in lens. The over-all level of activity in the tissues of immature animals is seen to be less than in the adult. By virtue of their developing state, certain areas of the lens and cornea increase while others decrease in enzymatic activity. Though these quantitative shifts do occur, there seems to be no major qualitative change in the enzymatic pattern of metabolism.

Summary

G6DH, ICDH, LDH, GDH, HK, and aldolase were assayed in corneal epithelium, stroma, and lens capsule, cortex, and nucleus of rats 0, 10, 20, and 100 days of age.

The activity of G6DH, ICDH, and LDH increases in corneal epithelium and decreases in stroma. Lens capsule plus epithelium accounts for most of the activity of lens, while lens nucleus has the lowest activity. The cornea is enzymatically more active than the lens. Hexokinase has a relatively greater prominence in immature developmental states than in later more mature states. The oxidative pathway appears to be prominent in corneal epithelium. While the capacity for it increases with age here, it decreases with age in stroma and lens. The implications of these findings are discussed.

Marked quantitative enzymatic changes follow development, but there seem to be no major qualitative shifts.

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EVALUATION OF THE "PHOSPHENATOR TEST"*

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Any procedure which is claimed to provide a simple, rapid, and atraumatic test for a common ocular disease, particularly where such a test is lacking, warrants an immediate evaluation. Such a claim was made for the "Phosphenator Test" as a means of detecting glaucoma.

The "Phosphenator Test" was first announced in a paper by C. E. Humphrey and V. J. Murgolo, appearing in the February, 1957, issue of the *Eye, Ear, Nose, and Throat Monthly*.[1] The test consisted of the passage of a pulsating electric current between two electrodes so placed on the head that some of the current passed through the eyes. This resulted in a sensation of light (called the electric phosphene), which flickered at the rate of the current's pulsations. The authors claimed that "a bluish-white phosphene is observed by the normal eye, while in the case of increased intraocular pressure, the patient reports yellow, orange, or red phosphenes." They also claimed that the color shifts toward the red end of the spectrum with increased intraocular pressure.

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On the same day that the paper was brought to our attention an apparatus was assembled with which to conduct the test. It consisted of a Heathkit Audio Oscillator to whose square wave voltage output was connected a pair of electrodes. Each electrode was simply a chlorided silver disk fitted inside a rubber cup (baby bottle nipple) which was then filled with electrode jelly. The electrodes were hung by their leads from a headband worn by the subject, who held the electrodes against the temporal margins of his orbits. These simple electrodes proved very serviceable and were used through the entire study.

Since no current value had been specified in the paper describing the test, in each case as low a value was used as would just permit unmistakable perception of the phosphene. In addition a 47,000 ohm resistor was placed in series with the ungrounded side of the oscillator's output, acting as a safety factor by limiting the average current between electrodes to a maximum of 0.5 ma.

TESTS ON GLAUCOMATOUS EYES

PROCEDURE

A first series of tests was run on outpatients at the eye clinic of The Ohio State University Hospital. In a semidarkened room the electrodes were applied, and the current turned on and increased until the subject reported a definite flickering light

TABLE 1

FIRST SERIES: CLASSIFICATION OF SUBJECTS

Category	Normal	Glaucoma	Other Pathology
Number of eyes	77	54	62
Average age (yr.)	35 estimate	64.0	46.6
Median age (yr.)	30 estimate	47.5	42

sensation in the peripheral field of vision. The subject was then asked to name the color of this flickering light.

Three categories of subjects were used in this first series of tests: patients with glaucomatous eyes, patients with eyes having other pathologies, and clinic staff and patients with normal eyes. A total of 193 eyes were tested in this series. The number of eyes tested and the average and median ages of the subjects are listed for each category in Table 1. As would be expected, the glaucomatous group was the oldest.

Phosphene colors. The results as to posphene colors reported are presented in Table 2. In accordance with the paper describing the "Phosphenator Test,"[1] colorless, white, gray, blue, and green phosphenes were classed as "normal" phosphene colors. Any color report from yellow green to red was classed as an "abnormal" phosphene color. In seven eyes no electric phosphene could be produced, even with the maximum current.

TABLE 2

FIRST SERIES: PHOSPHENE COLOR RESPONSES

Category	Normal	Glaucoma	Other Pathology	All
Phosphene Color	Number of Eyes			
"Normal"	66	45	48	159
"Abnormal"	11	6	10	27
Not seen	0	3	4	7
	% of Eyes in Category			
"Normal"	85.7	83.5	77.4	82.4
"Abnormal"	14.3	11.0	16.1	14.0
Not seen	0	5.5	6.5	3.6

"Normal" phosphene colors were defined as reports of colorless, white, gray, blue, or green.

"Abnormal" phosphene colors were defined as yellow, orange or red. Subjects who saw no phosphene at the maximum current strength are listed as Not Seen.

Two significant findings can be noted from these results:

1. Eighty-two percent of the glaucomatous eyes saw "normal" phosphene colors.

2. Fourteen percent of the normal eyes saw "abnormal" phosphene colors.

Relation of intraocular pressure and phosphene color. As part of the clinical routine, the intraocular pressure was determined with a corneal tonometer for some of the patients on the same day they were tested with the electric phosphene. These readings were later examined for a possible correlation between intraocular pressure and phosphene color. The results are shown in Table 3. As would be expected, the Glaucoma category had a significantly higher mean intraocular pressure than had the Other Pathology category. It was found that there was no statistically significant difference in mean intraocular pressures of the "normal" phosphene color group and the "abnormal" phosphene color group for either the Glaucoma category or the Other Pathology category.

A secondary finding was that the three eyes failing to get an electric phosphene had a significantly lower mean intraocular pressure than the rest of the Glaucoma category (9.4 mm. Hg less, at 0.5 percent significance level).

Another way of examining the data for a correlation between intraocular pressure and phosphene color is to separate the eyes into two classes: those having pressures in the normal range of 10 to 25 mm. Hg, and those having pressures of 25 or more mm. Hg. Table 4 shows this classification. It can be seen that most of the eyes with high intraocular pressures had "normal" phosphene colors, and many of the eyes with normal pressures had "abnormal" phosphene colors.

Relation of phosphene color to age. The data of this first series of tests were also examined for any correlation between phosphene color and the age of the subject. The

TABLE 3

FIRST SERIES: DISTRIBUTION OF MEAN INTRAOCULAR PRESSURE WITH CATEGORY AND PHOSPHENE COLOR, AND COMPARISONS OF THE MEANS

Category	Mean Intraocular Pressure (mm. Hg)			
	Whole Category	"Normal" Colors	"Abnormal" Colors	Not Seen
Glaucoma	24.36	25.13	24.17	15.0
Other Pathology	18.18	18.40	18.00	—

Groups Being Compared	Difference in Mean IOP (mm. Hg)	Occurrence by Chance	Statistically Significant
Glaucoma vs. Other Pathology	6.18	0.04%	Yes
Glaucoma, "Normal" vs. "Abnormal"	0.96	58%	No
Other Pathology, "Normal" vs. "Abnormal"	0.40	89%	No
Glaucoma, Not Seen vs. Whole Category	−9.36	0.5%	Yes

TABLE 4

FIRST SERIES: DISTRIBUTION OF PHOSPHENE COLOR WITH INTRAOCULAR PRESSURE

Category	Glaucoma		Other Pathology	
Intraocular Pressure	<25 mm. Hg	≥25 mm. Hg	<25 mm. Hg	≥25 mm. Hg
Phosphene Color	Number of Eyes			
"Normal"	17	21	5	0
"Abnormal"	3	3	6	0
Not seen	3	0	0	0
	% of Eyes in Category			
"Normal"	36.2	44.6	45.5	0
"Abnormal"	6.4	6.4	54.5	0
Not seen	6.4	0	0	0

results are shown in Table 5. No significant difference was found between the mean ages of the "normal" phosphene color group and the "abnormal" phosphene color group for the Other Pathology category. For the Glaucoma category the mean age of the "abnormal" phosphene color group was higher than that of the "normal" phosphene color group (69.7 years, as compared to 57.8 years) at the 0.01-percent level of significance.

TESTS ON THE GENERAL POPULATION

PROCEDURE

A second series of tests was run on a sample of the general population; chiefly volunteers from among delegates to an Ohio

TABLE 5

FIRST SERIES: DISTRIBUTION OF MEAN AGE WITH CATEGORY AND PHOSPHENE COLOR, AND COMPARISONS OF THE MEANS

Category	Mean Age (yr.)			
	Whole Category	"Normal" Colors	"Abnormal" Colors	Not Seen
Glaucoma	64.04	57.79	69.67	75.67
Other Pathology	46.58	45.44	46.00	61.00

Groups Being Compared	Difference in Mean Age (yr.)	Occurrence by Chance	Statistically Significant
Glaucoma vs. Other Pathology	17.46	<0.01%	Yes
Glaucoma, "Normal" vs. "Abnormal"	−11.88	0.01%	Yes
Glaucoma, Not Seen vs. Whole Category	11.63	<0.01%	Yes
Other Pathology, "Normal" vs. "Abnormal"	− 0.56	92%	No
Other Pathology, Not Seen vs. Whole Category	14.42	1.5%	Yes

TABLE 6

COLOR CHIPS USED FOR COMPARISON WITH ELECTRIC PHOSPHENE COLOR

Designation	Appearance	Manufacturers No.
A	Light gray	G7
B	Darker gray	M4
C	Light blue	I5
D	Light green	G8
E	Yellow-white	F9
F	Light yellow-green	D9
G	Yellow	K1
H	Light yellow-orange	G6
I	Yellow-orange	M3
J	Orange	M1
K	Orange-pink	L10
L	Light purple	K10

These chips are available as High Style Color chips from the Dean and Barry Company of Columbus, Ohio.

Lions Clubs state convention. The same electrical apparatus as before was used, but it was modified by putting a germanium diode in series with each electrode. This ensured current flow in one direction only. Preliminary experiments showed that the appearance of the phosphene was not affected by whether the current flowed from the left electrode to the right electrode or the right to the left. The diodes caused the current pulses to be peaked rather than square, and caused the phosphene to be more apparent at the currents used.

The tests were conducted in a normally lighted room. To eliminate any possible confusion in the use of color names, the subjects were instructed to choose which of 12 colored chips of paper (described in Table 6) most closely matched the color of the electric phosphene. The chips were displayed on a black background and viewed under a combination of incandescent lamp light and daylight from a window. The subjects were allowed to close their eyes as an aid in examining the phosphene.

For each subject a card was made out listing the name, address, age, date, eye troubles, and relatives with glaucoma. One examiner measured the intraocular pressures with a corneal tonometer. He noted on the card these pressures, the corneal diameter, the apparent depth of the anterior chamber, and whether the subject wore eyeglasses correcting for hyperopia or myopia. Another examiner at a distant part of the room supervised the electric phosphene test and noted on the card the color chips selected by the subject. For about half of the subjects the pressures were measured first, and the phosphenes first for the remainder.

Relation of phosphene color and sex. A total of 213 eyes were tested in this series; 35 from female subjects, and 178 from male subjects. The average, median, and modal ages for these subjects in total and grouped by sex are listed in Table 7. The phosphene colors selected by each group of subjects are compared in Table 8. It can be seen that there is no great difference between the male and female groups either in age distribution or in distribution of selected phosphene colors. Therefore, it was concluded that the electric phosphene test was not significantly affected by the subject's sex, and that it was valid to treat all the subjects as a single group.

Relation of phosphene color and intraocular pressure. All of the eyes were found to have normal intraocular pressures (25 mm. Hg or less). So it is noteworthy that 24 percent of these eyes had "abnormal" phosphene colors.

The claimed existence of a relation be-

TABLE 7

SECOND SERIES: DISTRIBUTION OF SUBJECTS

Category	Male	Female	All
Number of eyes	178	35	213
Average age (yr.)	44.1	45.2	44.2
Median age (yr.)	51	47.5	51
Modal age (yr.)	approx. 43	approx. 47	approx. 43

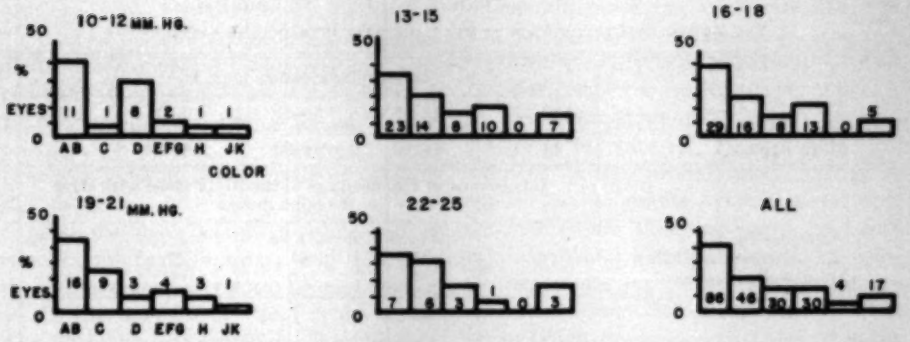

Fig. 1 (Lipetz). Second series, distribution of the electric phosphene color responses as a function of the intraocular pressure of the tested eyes. The number of eyes represented by each bar is given on or above the bar.

tween intraocular pressure and phosphene color was tested by observing the distribution of color for eyes of different pressures. This is shown in Figure 1 for a division of the eyes into five pressure ranges. It can be seen that there is a similar phosphene color distribution among the eyes for all of the five pressure ranges and for the total pressure range.

Furthermore, if all the eyes are divided into two groups according to whether they had a "normal" phosphene color (chips A, B, C, D) or an "abnormal" phosphene color (chips E through L), it is found that there is no significant difference between the mean intraocular pressures of the two groups (16.69 mm. Hg and 16.57 mm. Hg, respectively). So, from this finding and from the lack of change of color distribution with pressure (fig. 1), it is evident that there is no correlation between intraocular pressure and electric phosphene color.

Relation of intraocular pressure and age. In Table 9 the eyes are again divided into five groups according to their intraocular pressure, and the mean eye ages are shown for all groups. It can be seen that the mean ages of the pressure ranges are not significantly different. So it can be concluded that these subjects showed no significant relation between intraocular pressure and age.

Relation of phosphene color and age. The

TABLE 8

SECOND SERIES: PHOSPHENE COLOR DISTRIBUTION

Group	Number of Eyes							
	A, B	C	D	E, F, G	H	J, K	"Normal"	"Abnormal"
Male	68	44	22	24	3	17	134	44
Female	18	2	8	6	1	0	28	7
All	86	46	30	30	4	17	162	51
	% of Eyes in Group							
Male							75	25
Female							80	20
All							76	24

Colors A, B, C, D were classed as "Normal," colors E, F, G, H, I, J, K, L as "Abnormal." Colors I, L were not selected by any subject.

TABLE 9

SECOND SERIES: DISTRIBUTION OF MEAN AGE WITH INTRAOCULAR PRESSURE

	Intraocular Pressure (mm. Hg)				
	10–12	13–15	16–18	19–21	22–25
Mean Age (yr.)	42.84	44.10	44.80	45.56	43.15
	Comparison of the mean age of the 10–12 range with those of the other ranges				
Difference (yr.)		−1.26	−1.96	−2.72	−0.31
% Occurrence by Chance		60	41	29	90
Statistically Significant		no	no	no	no

subjects were divided into five age groups, and the distribution of phosphene colors within each group was examined. Histograms of these distributions are shown in Figure 2. It can be seen that the youngest age group had a larger proportion of eyes with A or B (gray) phosphene colors than had any of the other age groups.

The eyes were separated into six color groups according to the phosphene colors selected, and the mean age of the eyes determined for each color group. The mean ages are listed in Figure 2. The group selecting A or B was younger, at better than the 0.04-percent level of significance, than the group selecting D (by 6.3 years) or the group selecting E, F, or G (by 5.8 years). No valid comparison could be made with the groups selecting H or selecting J or K because of the small number of eyes in these groups. Furthermore, the combined group of A, B, or C is 5.2 years younger than the combined group of D, E, F, or G, and this is statistically significant at better than the 0.01-percent level.

This analysis indicates a definite tendency for the younger subjects to match the electric phosphene color with grays or blue.

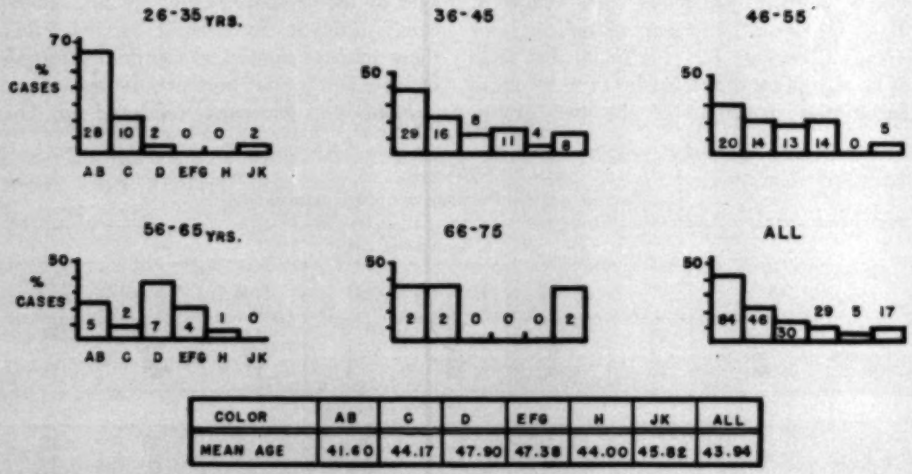

Fig. 2 (Lipetz). Second series, distribution of the electric phosphene color responses as a function of the age of the subjects. The number of eyes represented by each bar is given on or above the bar.

Discussion

Seven of the subjects in the second series of this study reported having relatives with glaucoma. This group of seven did not differ significantly in mean age, mean intraocular pressure, or percent of "abnormal" color responses from the whole group of subjects used in the series.

After the above study was completed, it was found that Humphrey and Murgolo used an average current of 1.5 ma. instead of the less than 0.5 ma. used in the study. The larger current causes the phosphene to spread more centrally into the visual field and to appear brighter, so that its color is more easily judged. A few subjects were retested with the stronger current, and reported the same phosphene color as with the weaker current.

The physiologic bases for the differences in perceived phosphene color are not known. This is a question worthy of investigation for its own sake, and it is to be hoped that someone soon undertakes it.

Another evaluation study of the "Phosphenator Test"[2] has recently been brought to my attention. It was conducted by Kurland, Davis, Sachs, and Kerpelman of the National Institute of Neurological Diseases and Blindness and the Walter Reed Army Hospital. Dr. Kurland has kindly furnished a copy of their manuscript. Their study was better conducted than this one in the following particulars: (a) They used apparatus and test conditions as specified by Dr. Humphrey; (b) they checked the subjects' color vision; (c) the examination of the subject, the determination of the intraocular pressures, and the determination of the phosphene color were done independently by different investigators.

They found that about three percent of the subjects gave an "abnormal" phosphene color response irrespective of the intraocular pressure. They concluded that the "Phosphenator Test" was of no value in detecting increased intraocular tension or in diagnosing glaucoma.

One difference in our procedures was that, on the advice of Humphrey, they had regarded a pale yellow phosphene color as "normal"; while I, in accordance with the paper by Humphrey and Murgolo, had regarded such colors (E, F, G, H) as "abnormal." If this revised color criterion is applied to the results of this study, then the number of "abnormal" color responses is reduced, as shown in Table 10. The overall occurrence of "abnormal" color responses becomes 5.2 percent in the first series (clinic testing) and 8.0 percent in the second series (general population testing), bringing the results of this study into much closer agreement with the study of Kurland, et al.

Another way of testing the importance of this color criterion difference on the results is to reclass the choice of pale yellow (their

TABLE 10

DISTRIBUTION OF PHOSPHENE COLOR RESPONSES BY CATEGORY, FOR PALE YELLOW (COLORS E, F, G, H IN SECOND SERIES) BEING CLASSED AS "NORMAL" RATHER THAN "ABNORMAL" RESPONSE

First Series

Category	Normal	Glaucoma	Other Pathology	All
Phosphene Color	Number of Eyes			
"Normal"	74	49	53	176
"Abnormal"	3	2	5	10
Not Seen	0	3	4	7
	% of Eyes in Category			
"Normal"	96.1	90.8	85.5	91.2
"Abnormal"	3.9	3.7	8.0	5.2
Not Seen	0	5.5	6.5	3.6

Second Series

Category	Male	Female	All
Phosphene Color	Number of Eyes		
"Normal"	161	35	196
"Abnormal"	17	0	17
	% of Eyes in Category		
"Normal"	90.5	100	92.0
"Abnormal"	9.5	0	8.0

chip number 3) as an "abnormal" response. The over-all occurrence of "abnormal" phosphene colors in their study then becomes 17.9 percent, much closer to the 14.0 percent of my first series and the 23.9 percent of my second series. So it can be concluded that the major part of the difference in results between the two studies is caused by the different criteria used for the "normal" and "abnormal" phosphene colors. Despite the differences in the testing procedures both studies reached the same major conclusions.

This evaluation of the "Phosphenator Test" was not carried out under the exact conditions finally specified by Humphrey and Murgolo. Nevertheless, the results of this study are in such disagreement with what has been claimed for the "Phosphenator Test" that the burden of proof is now on its proponents to show under what conditions, if at all, it is valid as a test of glaucoma and of intraocular pressure.

Conclusions

It can be concluded that under the test conditions used:

1. The color of the electric phosphene is no indication of the presence or absence of glaucoma.

2. The color of the electric phosphene is no indication of the amount of intraocular pressure.

3. There is some correlation between age and the color of the electric phosphene. This showed as a tendency for the older glaucomatous subjects to report a phosphene color toward the red end of the spectrum, and as a tendency for the younger normal subjects to report a gray or blue phosphene.

Department of Ophthalmology (10).

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HEALING OF ENDOTHELIUM AND DESCEMET'S MEMBRANE OF RABBIT CORNEA*

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AND

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Leber[1] (1873) was the first to point out the importance of the endothelium in corneal transparency, and Fuchs[2] (1917) was the first to suggest that Descemet's membrane was a product of endothelial secretion. Stocker[3] (1953), in experimental studies, showed that, following an injury to the endothelium and Descemet's membrane, the complete reconstitution of the latter structure did not occur, although a membrane thinner than the original appeared to be secreted by the new endothelium.

Cogan[4] has studied the regeneration of endothelium experimentally, and has shown that healing is rapid and complete in the presence of a normal Descemet's membrane. He could not demonstrate any morphologic or functional abnormality in the regenerated endothelium.

Our present studies were undertaken to

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gain further histologic evidence of endothelial healing and of the reconstitution of Descemet's membrane, with particular reference to the healing of corneal incisions in keratoplasty and cataract extraction.

Methods and Materials

Thirty rabbits were used in these experiments. Under general anaesthesia, a discission needle was introduced into the anterior chamber of the rabbit's eye at the superior limbus, and the inner aspect of the cornea was scarified with a single vertical stroke. The animals were then killed at intervals from three hours to 180 days. The corneas were sectioned, stained with eosin and hematoxylin, and examined microscopically. Control of the extent of trauma was achieved after practice. Photomicrographs were taken at various stages and measurements derived from these showed that the endothelial defect varied in width between 0.08 and 0.14 mm.

Examination of the histologic preparations revealed marked edema of the stroma within three hours, most prominent at the site of injury (fig. 1). Within six hours there were fibrin clots adherent to the denuded stroma, and scattered round cells were enmeshed in the clot (fig. 2). The first evidence of endothelial regeneration was seen after 24 hours, and by 48 hours there was a well-defined endothelial layer migrating over the defect from the cut and re-

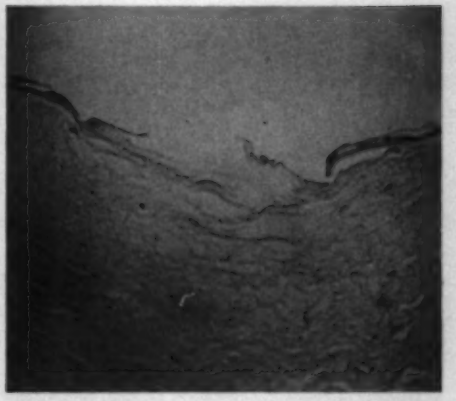

Fig. 1 (Morton, Ormsby, and Basu). Three hours after injury. Marked edema of the stroma.

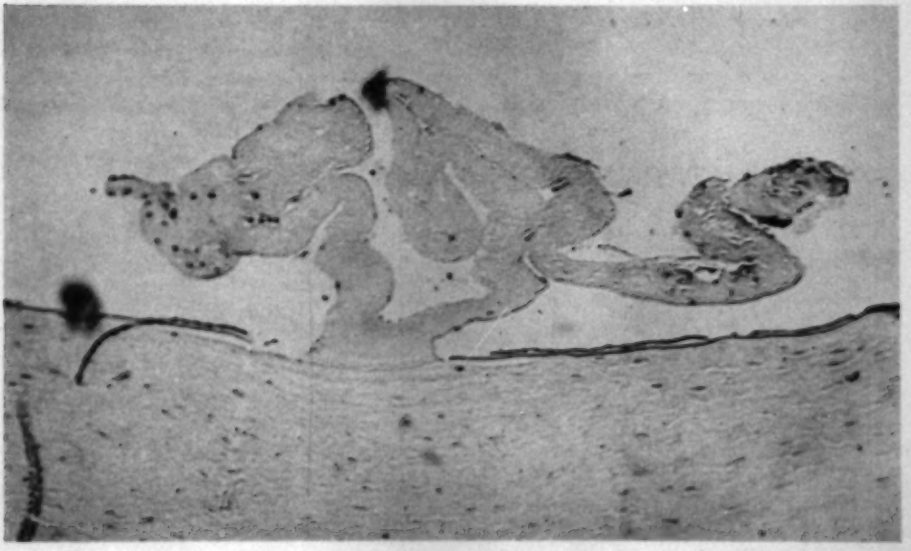

Fig. 2 (Morton, Ormsby, and Basu). Six hours after injury. Formation of fibrin clots.

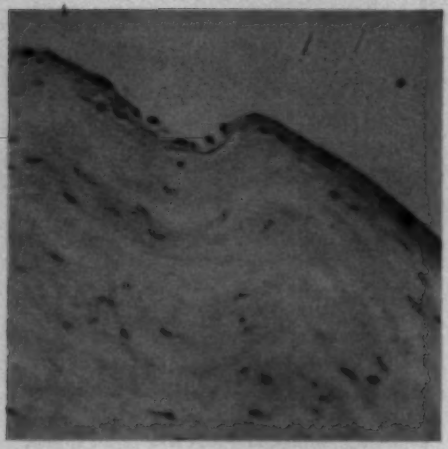

Fig. 3 (Morton, Ormsby, and Basu). Forty-eight hours after injury. Early endothelial migration over defect.

tracted Descemet's membrane (fig. 3).

As the endothelium was regenerated, the edema of the substantia propria subsided. Loose fragments of Descemet's membrane showed growth of endothelium on both surfaces in sections cut at three days (fig. 4).

Generally, the growth of endothelium was prolific, for it did not stop when the defective area had been filled, but continued to proliferate until a fusiform scar of cellular tissue had been produced over the area of trauma (figs. 5 and 6). It was not possible to determine whether this "scar" was derived entirely from endothelial cells, since there was some evidence of cellular activity in the underlying stroma, but the relatively small contribution of the stroma to the scar formation is suggested by the close approximation of the scar to the surrounding endothelium.

Between one and two weeks after trauma, there was a gradual hyalinization of the scar, proceeding from the underlying substantia

Fig. 4 (Morton, Ormsby, and Basu). Three days after injury. Endothelial proliferation on fragments of Descemet's membrane.

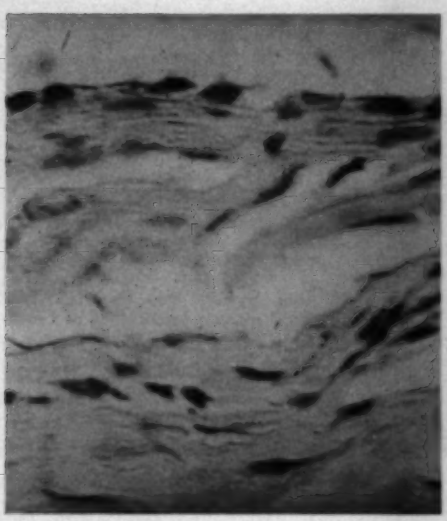

Fig. 5 (Morton, Ormsby, and Basu). Five days after injury. Endothelial "scar" over cut edge of Descemet's membrane.

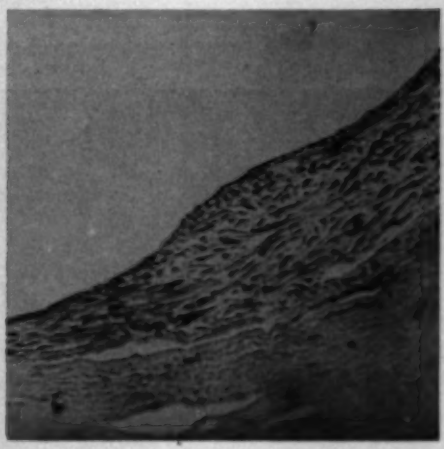

Fig. 6 (Morton, Ormsby, and Basu). Eight days after injury. Advanced endothelial "scar."

toward the endothelial surface. This hyalinization and metamorphosis of scar into stroma-like tissue continued for three weeks, at which time all that marked the site of the original area of trauma were the absence of Descemet's membrane and slight increase in subendothelial cellularity in this area.

The time at which Descemet's membrane began to reappear could not be accurately determined. It was questionable whether it could be seen at any time before 21 days, but it was constantly present after that time. The regeneration was slow, however, and at 90 days Descemet's membrane had developed to about 50 percent of its original thickness. Subsequent observations up to 180 days failed to show any further increase in thickness of the new Descemet's membrane (figs. 7 to 11). In staining characteristics, the new membrane was comparable to the old, except for a slightly paler hue.

Fig. 7 (Morton, Ormsby, and Basu). Thirteen days after injury. Hyalinization of endothelial "scar."

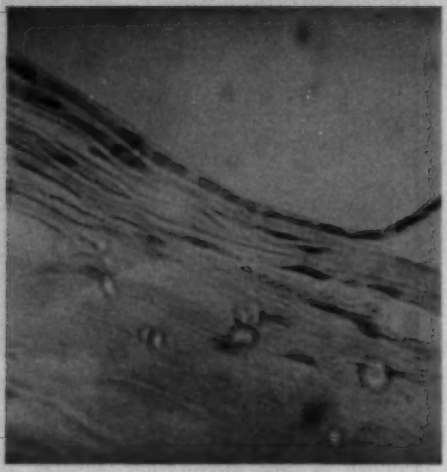

Fig. 8 (Morton, Ormsby, and Basu). Twenty days after injury. Beginning regeneration of Descemet's membrane.

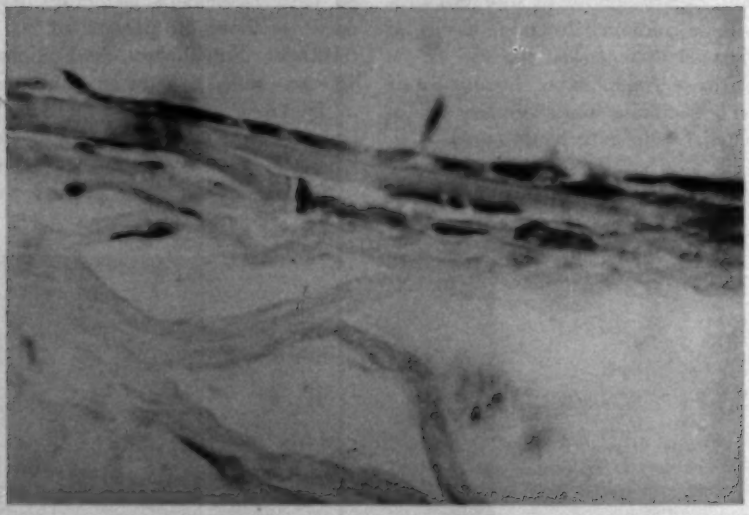

Fig. 9 (Morton, Ormsby, and Basu). Ninety days after injury. Regeneration of Descemet's membrane to 50 percent of its original thickness.

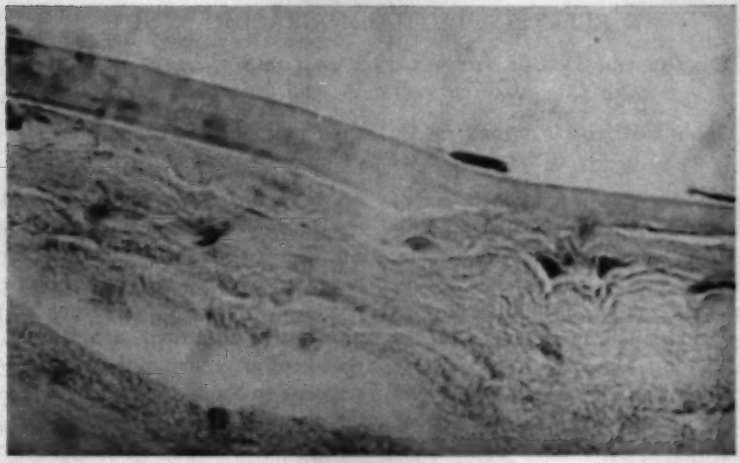

Fig. 10 (Morton, Ormsby, and Basu). One hundred eighty days after injury. Regeneration of Descemet's membrane is no more than that found at 90 days.

The surface of the new Descemet's membrane adjacent to the endothelium was smooth, but the surface adjacent to the stroma was irregular, and showed no sign of absorption.

Discussion

Our observations confirmed those of previous workers in that the endothelium regenerated rapidly. The excessive proliferation of the scar may have been due either to the regenerative power of the endothelium or to an abundant contribution to the scar from the substantia propria. Morphologically, it was difficult to distinguish between cellular connective tissue and adjacent endothelium. Whatever the origin of the cells which repaired the defect, they lacked the ability to produce more than 50 percent of the original thickness of Descemet's membrane. It may be that the formation of a normal, thick membrane is a long-term process, but this was not evident in these experiments.

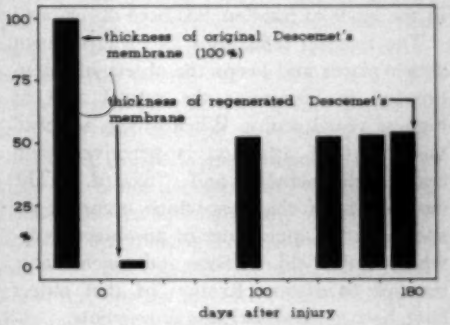

Fig. 11 (Morton, Ormsby, and Basu). Graph of regeneration of Descemet's membrane in rabbit cornea.

Banting Institute.

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AN ELECTRO-OPHTHALMOGRAPHIC STUDY*

Of the behavior of the fixation of amblyopic eyes in light- and dark-adapted state: A preliminary report

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The ability for steady fixation is not as good in eyes with reduced visual acuity as it is in eyes with normal vision. One should, therefore, expect eyes with strabismic amblyopia not to fixate as steadily as do eyes with standard vision, or the sound eye of the same person.

The fixation pattern of amblyopic eyes has received of late years a great deal of attention, particularly in the European literature. The mode of fixation has been correlated with the visual acuity and a new form of treatment, based on an evaluation of the mode of fixation, has been developed.[1]

The fixation reflex acts as an adjustment which places and keeps the object of attention on the fovea as the retinal area of highest visual acuity. When this is achieved the object of attention is perceived with optical distinctness and "fixated." The movements of the eyes which occur in response to the appearance of an object somewhere in the field of vision and which enable the eye to assume fixation of that object have been termed saccadic movements.

Electro-ophthalmography provides a valuable method for the registration and analysis of both the fixation movements and the saccadic movements of the eyes. This method is based on the fact that there exists an electrical potential between the cornea and the posterior pole of the globe. This resting potential is not affected by eye movements, but eye movements change the relationship of the electrical field of the eyes to fixed electrodes placed somewhere near the globe. Such electrodes will pick up positive or negative charges which, when properly amplified and recorded, will result in positive or negative deflections, according to the direction of movement of the eyes. The graphic response so obtained is usually referred to in the literature as an electro-oculogram, but we prefer to call it electro-ophthalmogram (EOG) to avoid an unnecessary hybrid formation.

The electro-ophthalmographic behavior of the fixation movements and the saccadic movements of amblyopic eyes have been the subject of two recent studies by Mackensen.[2,3] This author has reported disturbances of the fixation ability of amblyopic eyes ranging from oscillating movements to jerky fixation movements, whereas the sound eye always remained steady within the limits of the recording method.

To learn more about strabismic amblyopia, and particularly about the quite extraordinary ability of the amblyopic eye for steady fixation in dark adapted state,[4] we undertook at the suggestion of one of us (H. M. B.) a comparative electro-ophthalmographic investigation of light- and dark-adapted amblyopic eyes.

Materials and Methods

Nine patients with an amblyopia of one eye ranging from 6/12 to 6/60 were examined. All had a comitant esotropia and the amblyopia was typical of squinters; that is, none of the amblyopic eyes exhibited any observable pathologic changes. Clinically the fixation behavior of these patients varied from "central fixation" to "wavering fixation." This was determined simply by

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observation of the light reflexes from the cornea. The photographic method of Steiger and Würth[5] which makes use of the fundus camera provided with special fixation mark appearing on the fundus photograph was tried out by us. This method allegedly differentiates between foveolar, parafoveolar, eccentric, and wavering fixation, but we did not find it to be of the desired accuracy and the method needs improving. We are at present engaged in attempting such an improvement and shall report on our results on another occasion.

In addition to the patients with strabismic amblyopia we also made electro-ophthalmographic studies in a patient with an amblyopia due presumably to trauma to the optic nerve and on a patient with an old central chorioretinitis.

The apparatus used in this study was patterned after the one employed by Mackensen. It consisted of a black horizontal arc with a radius of one meter carrying five-degree marks and extending 45 degrees to each side of the center (fig. 1). Three ophthalmoscope bulbs, enclosed in square housings, could be placed at any desired position on the arc. The lamps could be separately activated from a central control box containing transformers and rheostats and a circuit permitting to record the moment of switching on and off of the lamps.

Silver cup electroencephalographic electrodes were fastened to the patient's skin at

Fig. 1 (von Noorden and Burian). Fixation device. For explanation see text.

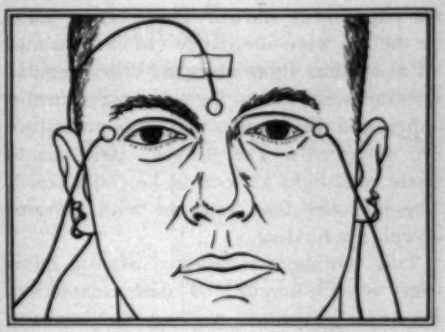


Fig. 2 (von Noorden and Burian). Electrode placement for electro-ophthalmography.

the outer corner of each eye and to the bridge of the nose (fig. 2); ground electrodes were placed on the lobes of each ear.

The recording was done with a Grass 4-channel inkwriting electroencephalograph. The voltage differences between the two temporal leads were recorded on one channel, thus registering the movements of the two eyes simultaneously. The movements of the right and left eye were separately recorded on two additional channels. In principle this is not necessary and the recording from both eyes simultaneously should suffice since the nonfixating eye follows the movements of the fixating eye quite faithfully. However, our arrangement permitted us to differentiate eye movements from lid movements which appeared in all three channels as low voltage positive deflections.

The onset and the cessation of each stimulus were recorded on the fourth channel.

Experimental procedure

1. Fixation movements

One light source was placed in the center of the arc, the second one 25 degrees to the left, and the third one 25 degrees to the right. All bulbs were covered with a two-mm. pinhole screen. After occluding the amblyopic eye the patient was seated comfortably at one m. distance from the central fixation light. He was then light adapted for two min. by means of a 500-watt bulb and

the experiment started. The fixation bulbs on the arc were alternately switched on and off at random time intervals. When regular intervals were chosen the patients frequently anticipated the stimulus and made premature eye movements. The patient's task was to fixate each light as soon as he could see it. The test was then repeated with the amblyopic eye fixating.

This routine was repeated after a minimum of 25 minutes of dark adaptation. Rheostats permitted to keep the intensity of the fixation lights at a minimum to guarantee and maintain good dark adaptation.

2. Saccadic movements

Only two lights were used in this test. One served as a fixation light at 25 degrees to the left of the center and remained during the entire experiment. The front of the housing of this light was supplied with a filter in the form of a red cross to provide easy fixation. A white cross could be flashed on and off at 25 degrees to the right of the center. The patient was light adapted and occluded in the same way as described above. He was told to fixate the red cross at his left and only move his eyes to fixate the white cross when the latter was flashed on. This was also done at arbitrary time intervals ranging from five to 25 seconds. The same test was then repeated with the amblyopic eye fixating, and both eyes were examined in the same manner after dark adaptation.

Results

When the normal eye assumes fixation it does so with one simple movement, and thereupon maintains its position rather steadily. This is clearly seen from the electro-ophthalmograms of normal eyes (fig. 3). However, it must be pointed out that the electro-ophthalmographic method in its present form is not sensitive enough to register the fine fixatory oscillations which are known to exist normally and that it records only relatively

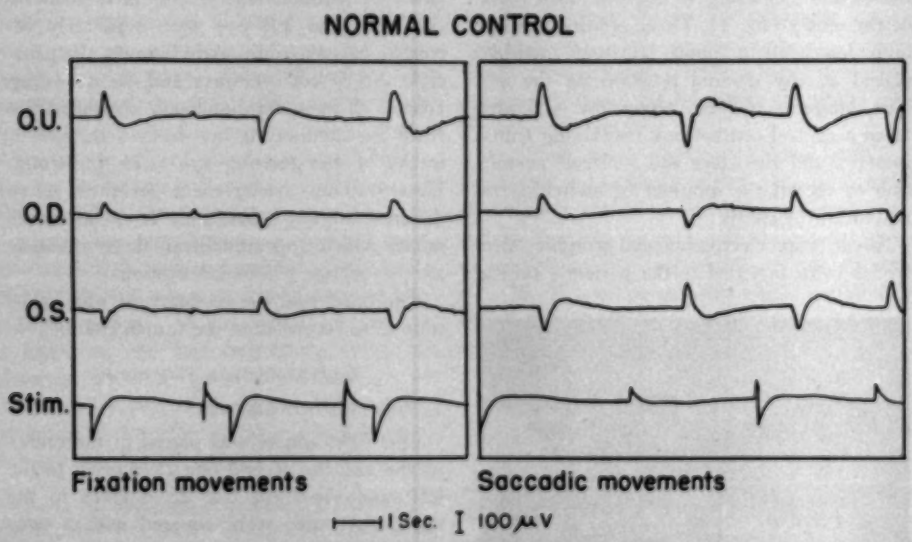


Fig. 3 (von Noorden and Burian). Electro-ophthalmogram of patient with normal eyes. Channel 1: recording from bitemporal leads. Channels 2 and 3: recording from the right and left eye, respectively. Positive deflections in channel 1 and channel 2 indicate movement to the right, negative deflections indicate movement to the left. The opposite holds true for channel 3. Channel 4: negative deflection, light on; positive deflection, light off. The difference in amplitude in the channels has technical reasons and does not represent differences in movement.

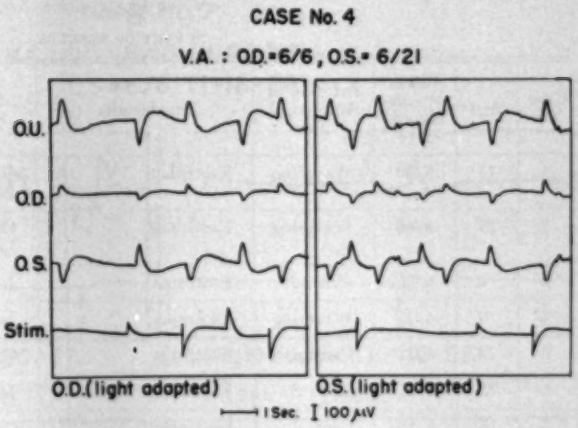


Fig. 4a (von Noorden and Burian). Fixation under standard conditions of light adaptation. Steady fixation with normal eye (O.D.) fixating, unsteady movements with amblyopic eye (O.S.) fixating.

coarse movements. Also, with the electrode placement employed by us horizontal movements alone were registered.

Within these limitations our electro-ophthalmographic study has yielded some very interesting results. When one compares the electro-ophthalmograms of normal eyes with those of light-adapted amblyopic eyes a pronounced difference becomes evident. In contrast to the normal eyes the fixation of the amblyopic eyes is characterized by great unsteadiness. In all cases the electro-ophthalmograms registered movements ranging from oscillations and nystagmus to wild, jerky movements (figs. 4a and 4b). Six of our nine patients showed jerky fixation movements when the amblyopic eye fixated, while three presented oscillating movements. In addition, two patients had latent nystagmus and one had a course fixatory nystagmus.

We have not attempted in this preliminary investigation to make a quantitative study of the unsteadiness of the fixation of the amblyopic eyes. It would appear, nevertheless, that there is no definite relationship between the degree of unsteadiness of fixation and the visual acuity. This may be seen from

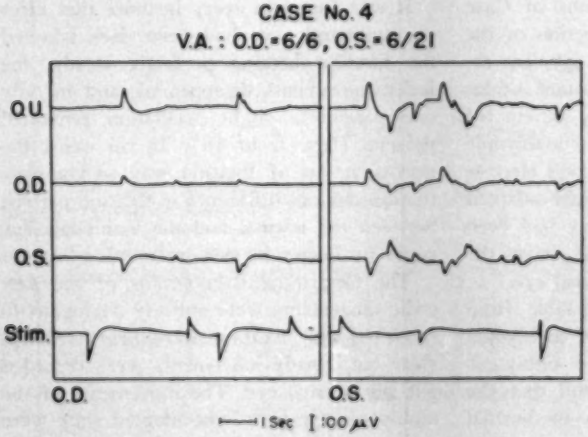


Fig. 4b (von Noorden and Burian). Saccadic movements under standard conditions of light adaptation. Immediate and steady fixation when normal eye (O.D.) assumes fixation of an object whose image appears in the retinal periphery. Unsteadiness and uncoordinated jerky movements when the amblyopic eye (O.S.) performs a similar movement.

TABLE 1

SURVEY OF RESULTS

Case No.	Age	Vision of Amblyopic Eye	Fixation of Amblyopic Eye	Strabismus	EOG of Amblyopic Eye (light adapted)	EOG of Amblyopic Eye (dark adapted)
1	11	6/60	Wavering	Esotropia	Jerky movements Latent nystagmus	Oscillating
2	29	6/60	Wavering	Esotropia	Oscillating movements Latent nystagmus	Steady
3	6	6/30.	Central	Esotropia	Jerky movements	Steady
4	9	6/21	Wavering	Esotropia	Jerky movements	Steady
5	28	6/21	Central	Esotropia	Oscillating movements	Steady
6	20	6/21	Central	Esotropia	Jerky movements	Steady
7	16	6/15	Central	Esotropia	Oscillating movements Fixation nystagmus	Steady
8	9	6/15	Central	Esotropia	Jerky movements	Steady
9	6	6/15	Central	Slight esotropia, monocular astigmatism, amblyopia ex anopsia	Jerky movements	Steady
10	10	6/60	Eccentric	Esotropia (paralytic), history of trauma to optic nerve	Jerky movements	Jerky movements
11	39	3/60	Eccentric	No strabismus, old central chorioretinitis	Oscillating	Oscillating

Table 1. While there may not be such a direct relationship, the fixation pattern of the amblyopic eye does return to normal with improvement of its acuity. This is strikingly demonstrated by Figure 5, in which the electro-ophthalmograms of Case 9, taken before and after correction of the visual acuity of the amblyopic eye, are reproduced for comparison. Constant occlusion of the fixating eye of this patient led to an increase of the acuity of the amblyopic eye from 6/15 to 6/6. The second electro-ophthalmogram taken in the light adapted state after standard visual acuity had been recovered was indistinguishable from the electro-ophthalmogram of a normal eye.

It was to some extent predictable from what is known about strabismic amblyopia that the fixation pattern of the amblyopic eye would behave erratically and that the fixation pattern would return to normal after the restoration of standard visual acuity. The most important result of this study, relating to the behavior of the fixation pattern of the dark adapted amblyopic eye, was much more surprising.

It was found in every instance that after the amblyopic eye had been dark adapted the fixation became perfectly steady, the jerky movements disappeared, and in only one patient a slight oscillation remained present. (figs. 6 to 10). In all cases the normalization of fixation was so complete that an obvious difference in fixation pattern between the normal and the amblyopic eye could no longer be demonstrated.

The electro-ophthalmograms of the saccadic movements were entirely analogous to those of the fixation movements. Sharp, clear cut, steady movements were recorded for the normal eye. The movements of the amblyopic eye in a light-adapted state were

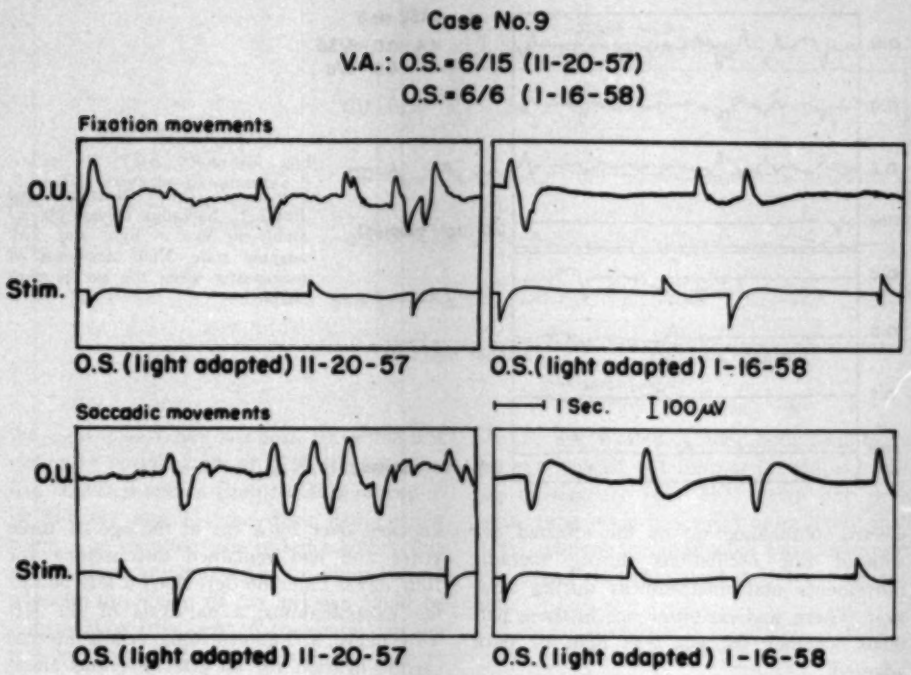


Fig. 5 (von Noorden and Burian). Recovery of steady fixation in fixation movements and saccadic movements after restoration of visual acuity.

jerky and irregular, but in the dark adapted state the movements of the amblyopic eye resembled again closely those of the normal eye.

For purposes of comparison we recorded the electro-ophthalmograms of the eyes of a 39-year-old man who had an old inactive chorioretinitis involving the macula of one eye. This eye had a visual acuity of 3/60; the other eye had a visual acuity of 6/6. The

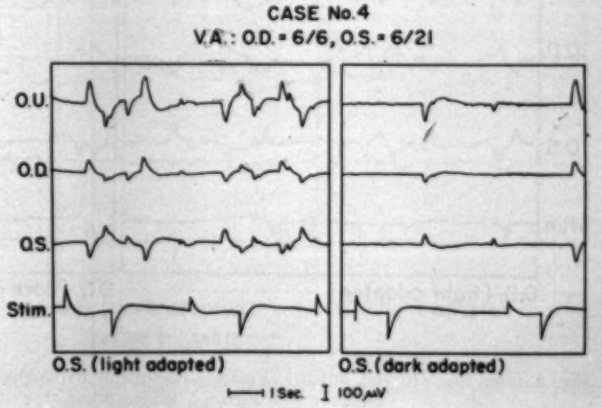


Fig. 6 (von Noorden and Burian). Fixation movements of amblyopic eye in light and dark adapted state. Note recovery of steady fixation in dark adaptation.

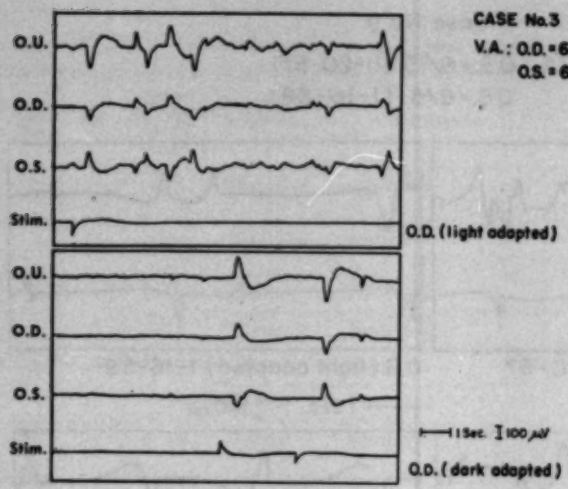


Fig. 7 (von Noorden and Burian). Saccadic movements of amblyopic eye in light and dark adapted state. Note steadiness of movements when the eye is dark adapted.

electro-ophthalmogram of the affected eye showed fine oscillations during saccadic movements and unsteadiness during fixation. There was no difference in these patterns whether the eye was light or dark adapted.

Another case presenting special features was that of a 10-year-old boy who had been knocked over by a car at the age of three years and had remained unconscious for four days. Later he developed a left pulsating exophthalmos, a paralysis of the left VIth nerve, and an esotropia. A left internal carotic ligation for an arterio-venous aneurysm was carried out. The exophthalmos improved as did the abducens paralysis and

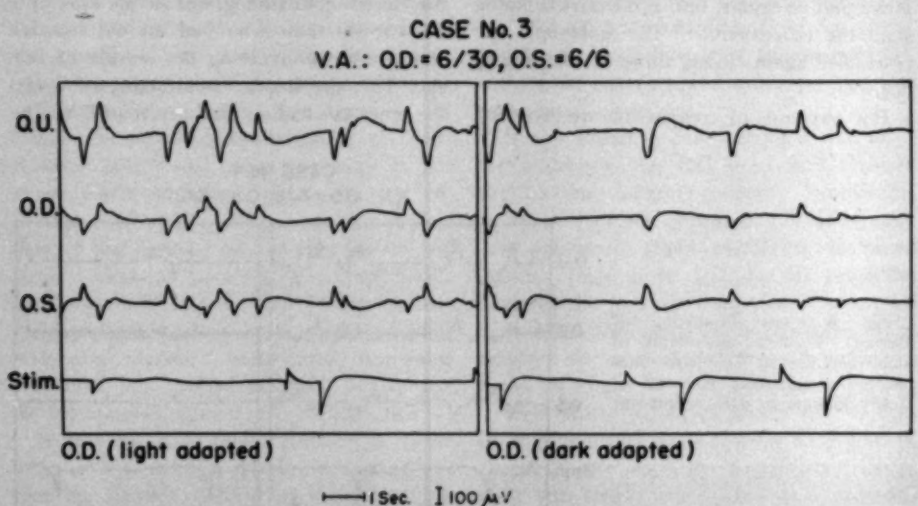


Fig. 8 (von Noorden and Burian). Fixztion movements of amblyopic in light and dark adapted state. Compare with Figure 5 demonstrating result of treatment.

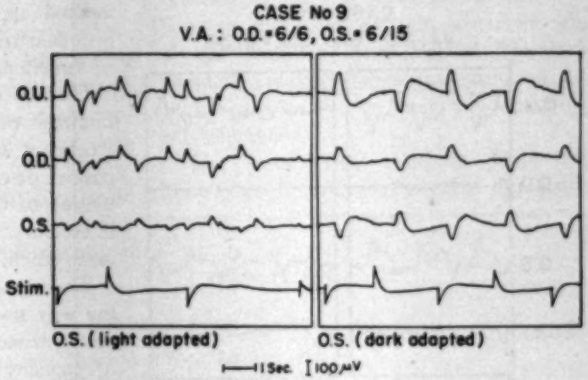
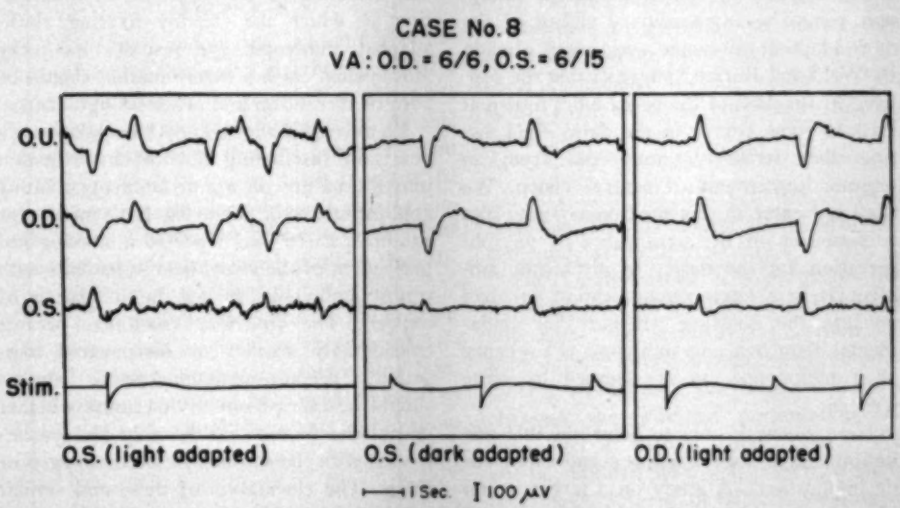


Fig. 9 (von Noorden and Burian). Saccadic movements of amblyopic eye in light and dark adapted state. Same patient as in Figure 8.

the esotropia. When last seen by us the left eye had a visual acuity of 6/30, the right eye had standard vision. Both fundi appeared to be entirely normal; the discs had normal color. The question was raised whether this patient had an amblyopia ex anopsia and whether the vision of his left eye could be improved by treatment. A traumatic damage to some part of the visual pathways had to be excluded. In this patient there was no difference in the electro-ophthalmograms in light-and-dark adapted state when the amblyopic eye fixated. Under both conditions the eye carried out unco-ordinated, searching movements (fig. 11). From our previous experience it seemed more likely that in this patient the amblyopia was organic in origin and that treatment would not benefit him.

Comments

The results of our investigations have confirmed the usefulness of the electro-ophthalmographic method for the study of the



Fog. 10 (von Noorden and Burian). Saccadic movements. Note similarity between dark-adapted amblyopic and light-adapted normal eye.

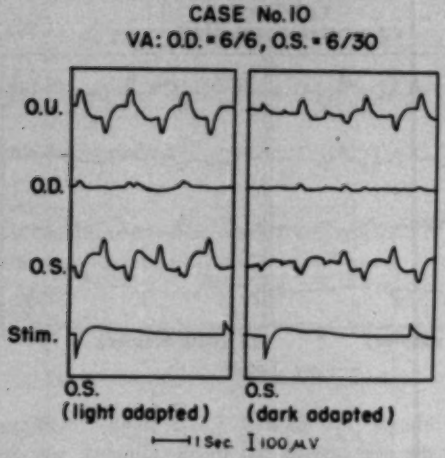


Fig. 11 (von Noorden and Burian). Fixation movements of an eye which has presumably an amblyopia of traumatic origin. Note unsteady fixation in both light- and dark-adapted state.

fixation pattern of eyes with strabismic amblyopia. They have also confirmed the expected unsteadiness of this pattern reported by Mackensen.[2,3]

In addition this investigation has given direct graphic evidence for the recovery of steady fixation by the dark-adapted amblyopic eye. The extraordinary ability of the dark-adapted amblyopic eye, noted already by Wald and Burian,[4] suggests that the suppressed function of the fovea must return at least to some degree in the dark. This distinguishes strabismic amblyopia from an organic impairment of central vision. We shall not enter in this preliminary note into a discussion of the significance of this observation for the theory of strabismic amblyopia. In a later communication we shall go into the question whether the fundamental disturbance in amblyopia is a sensory or a motor one, as is assumed by some writers

Here we wish only to point out that our findings have both diagnostic and therapeutic implications. A study on a larger group of patients which is now in progress will decide whether the electro-ophthalmographic method applied to light and dark adapted eyes is a reliable tool for the differentiation of functional from organic amblyopia.

Certain therapeutic applications of our findings can suggest themselves. Modern treatment of squint amblyopia consists of passive or combined passive and active stimulation of the fovea in cases where fixation is eccentric, and of active foveal training in cases where fixation is central.[1]

The knowledge gained in our study points the way toward an extension of our therapeutic armamentarium. The foveola resumes its capacity for steady fixation in the dark also in eyes with wavering or eccentric fixation which are unable to utilize their foveola under standard light conditions. Macular training in severely restricted illumination after thorough dark adaptation, with gradually increasing illumination under constant electro-ophthalmographic control of fixation, should be the therapeutic approach making use of the functional improvement of the foveola in dim illumination. Suitable instrumentation permitting such treatment is being tried out at this time.

A corollary to this therapeutic study is the determination of the threshold of illumination at which the steadily fixating, dark-adapted amblyopic eye resumes its jerky movements. Such a determination should be both of theoretical and practical significance.

Electro-ophthalmography has opened up a new and fascinating field of investigation into the nature of squint amblyopia. Many applications come immediately to mind. For example, Bietti[6] has reported a striking improvement of the visual field in patients with squint amblyopia during the inhalation of oxygen. The functional scotomas became considerably smaller or disappeared altogether. Electro-ophthalmographic studies should be carried out to determine whether or not the fixation of the ablyopic eye improves with the administration of oxygen or drugs. The elucidation of these and similar questions should be greatly assisted by use of the electro-ophthalmographic method.

Summary

The fixation movements and the saccadic eye movements of nine patients with squint amblyopia and of two patients with organic amblyopia were studied electro-ophthalmographically in conditions of light and dark adaptation.

The light-adapted amblyopic eyes were characterized by unsteadiness and jerky movements during fixation, while the sound eyes fixated steadily.

Unsteadiness and jerky movements were seen to disappear entirely when the amblyopic eyes were dark adapted. The dark-adapted amblyopic eyes thus behaved like sound eyes as far as their fixation ability was concerned.

The possibilities resulting from this knowledge for future research, differential diagnosis, and therapy are pointed out.

University Hospitals.

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CORRECTION FOR THE GUESSING BIAS IN THE LANDOLT RING TEST*

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Introduction

When the Landolt ring is used for measuring acuity, there is a size below which a certain number of the rings which are called correctly, are so called by guessing. At this level the frequency that the break is called to the right, left, up, or down is apt to be influenced by the fact that the ring is like the letter C when the break is to the right and by other factors which can bias the calling of different positions of the break.

In a previous study we made, the relation of astigmatism to visual acuity was investigated without allowing for this calling bias. The present study has been made to show to what extent a calling bias exists and to show how a correction can be made for it.

In the previous study it was assumed that at the level of guessing the subject would call the breaks up, down, right, and left with equal frequency. In the present study an actual count of these frequencies has been made.

Method

Two emmetropic observers viewed Landolt rings at 18 feet monocularly through a three-mm. artificial pupil and a +1.5D. sph.

The Landolt rings of a given size were presented in four positions, up, down, left, and right for seven-second periods with three-second intervals between presentations during which time the ring position was changed, the order of presentation being scrambled in such a manner that it could not be repeated until 120 presentations had been made. A series of 60 such presentations involving the same size but different posi-

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tions constituted a "run." The experiment was repeated with rings of different sizes, 20/15, 20/20, 20/25, 20/35, 20/45, and 20/60.

Each subject made eight runs with each size ring, that is, 480 presentations for each size.

At each presentation the subject pressed one of four keys to indicate where he saw or guessed the break to be. If his response was correct this was recorded by the electric counter assigned to this position of the break. At each presentation the supervisor recorded manually which button the subject pressed, whether correct or incorrect.

Hence at the end of each run the operator knew the number of times the ring was presented with the break at each position, the number of times each position was called and the number of times each position was called correctly.

The black Landolt rings were presented at the center of a white square field (5.6 degrees in each direction) having a brightness of 80 foot-Lamberts.

Results

An equation has been devised that permits the calculation of the number of correct responses actually seen when allowance had been made for guessing. This equation can be applied in turn to the up, down, right, and left calls. If we accept that

$$N_c = N_s + (\bar{N} - N_s)\ \tfrac{1}{4} \qquad (1)$$

where for a given direction $\bar{N}$ is the number of times the position is called, N_c the number of times called correctly, N_s the number of times seen and $\tfrac{1}{4}$ is the guessing level of a four position ring, then

$$N_s = \frac{4N_c - \bar{N}}{3} \qquad (2)$$

The raw and corrected data for calls to the right, up, down and left for two subjects are shown in Figure 1. In the case of W. K. V. only two runs instead of eight were made for the 15-foot rings, and only six runs were made for the 20-foot rings. In order to compensate for this, the scores have been multiplied by the appropriate factors to keep all of the data expressed in terms of eight runs.

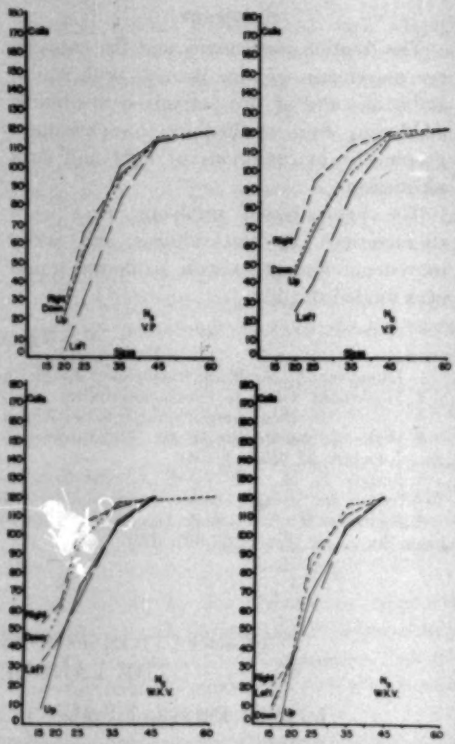

Fig. 1 (Prince and Fry). The raw scores (N_c) for two subjects, and the corrected scores (N_s) for the same subjects shown for all four positions of the Landolt ring test. It will be seen that the curves of N_s are much closer together than those for N_c.

It may be seen in Figures 2 and 3 that the number of incorrect calls varies greatly between the positions of the breaks, and that in the position "Break to the right" and to a somewhat less extent in the position "Break down" the number of calls greatly exceeds the number of presentations. This obviously accounts for the much lower number of calls for the "left" and "up" positions.

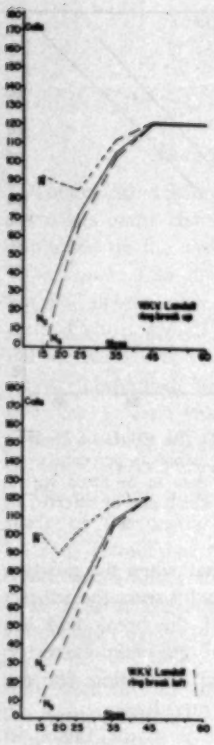

Fig. 2

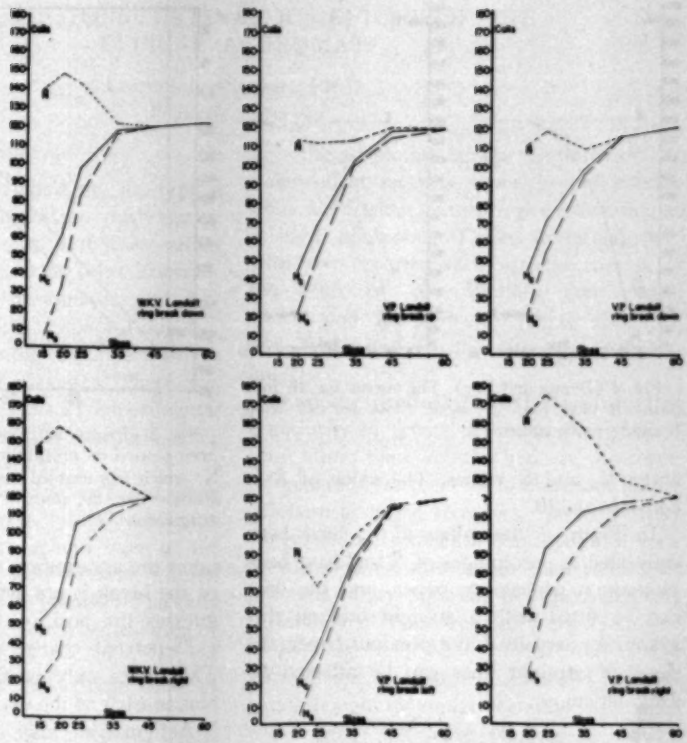

Fig. 3

Figs. 2 and 3 (Prince and Fry). These graphs show how greatly the number of incorrect calls varies between the break positions. In the right and down positions the number of calls greatly exceeds the number of actual presentations.

In this method of comparing the corrected and the uncorrected data, it may be noted that the corrected curves for "up" and "down" are closer together than the uncorrected curves, and the same holds true for the right and left data.

The compensation, however, is only partial. The differences for the different meridians can be attributed to astigmatism, but even after correction we still find differences for the two ends of the same meridian.

One kind of bias which has not been allowed for is the possibility that the subject may not be able to tell a right break from a left break even though he may be able to see that the break is neither up nor down.

In Figure 4 the curves for "up," "down," "right," and "left" have been averaged. If one is not interested in the factors that bias the four position responses, one can work directly from the data without reference to the position of the break. In this case, one is interested only in the number of *correct* responses, and the number seen. It is not necessary to total the number of responses because this is equal to the total number of stimuli. The equation for frequency of seeing (equation 2) is simplified as a result of the total number of responses being fixed from run to run.

The curves in Figure 4 for N_s can be computed by using equation 2, and the av-

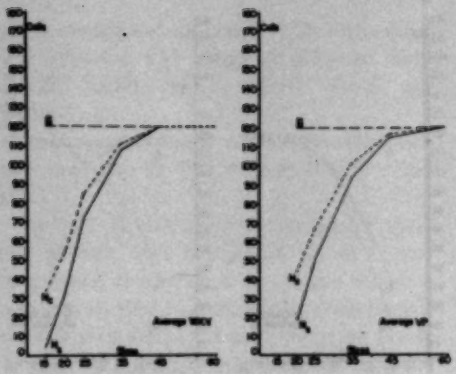

Fig. 4 (Prince and Fry). The curves for all four positions have been averaged, those for N_s being found by using equation 2.

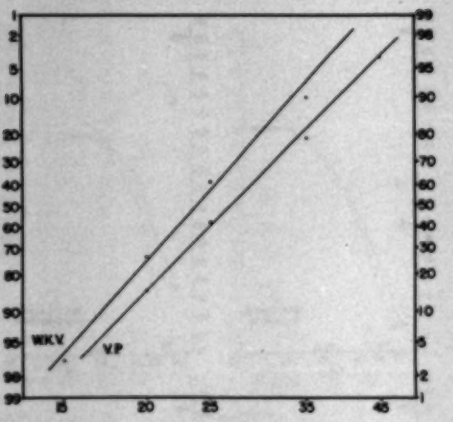

Fig. 5 (Prince and Fry). The values of N_s have been plotted on probability paper as percentages of $\bar{N}$, which has enabled the data to be fitted with a straight line, the slope of which can be affected by astigmatism.

erage N_s and $\bar{N}$ values. The value of $\bar{N}$ is constant at 120.

In Figure 5 the values of N_s have been converted to percentages of $\bar{N}$ and have been plotted on probability paper, and the data can be fitted with a straight line on this graph. As explained in a previous paper, the slope of straight lines can be affected by astigmatism.

Summary and Conclusions

The experiment has revealed that when a Landolt ring is not clearly visible, each of the two subjects used is more likely to guess the break as being to the right than to the left. This bias may be due to the likeness of the Landolt ring to the letter C when the break is to the right. No other cause for the bias has been identified. One of the subjects also shows a bias for "down" guessing over "up" guessing.

An equation for correcting data for such biases has been presented which is based upon the assumption that when the position of the break is not actually seen, the subject guesses the position of the break and has a 25-percent chance of guessing correctly. This gives only partial correction for the bias in each of the two meridians.

Astigmatism may be a major factor in producing differences between the two meridians of the Landolt ring when astigmatism does in fact exist.

The assumption of a 25-percent chance of guessing correctly is no doubt an oversimplification, because there are instances in which the subject can detect that the break is not "up" or "down," but cannot tell whether it is "left" or "right." This pattern of response can occur when astigmatism is present.

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THE PHOTOPIC RETINAL RECEPTORS OF THE TYPICAL ACHROMAT*

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INTRODUCTION

Congenital achromatopsia in its typical form has many characteristics which can be explained on the assumption that the retina lacks cones. The absence of color discrimination, photophobia, nystagmus, and low central acuity are all to be expected in a visual mechanism containing a normal rod receptor mechanism but no cones. There are, however, at least two lines of experimental evidence which suggest that receptors other than normal rods are present.

The first of these is Larsen's histologic study of the retina of a typical achromat. Larsen[1] found that throughout most of the retina cones and rods were present, normal both in structure and in number. The only abnormality observed was in a central area of a few degrees where both the cones and the rods were shorter and thicker than those of the normal retina.

A second type of evidence for the existence of receptors other than normal rods is provided by measurement of the rate of dark adaptation after pre-exposure to a high luminance. In the conventional graph, showing change in log threshold with time in the dark, the normal eye shows first a rapid drop in threshold then a plateau, followed by a second drop and a second plateau. The generally accepted explanation for this bipartite curve is that the first section is determined by the cone thresholds, the second by those of the rods.

In a previous study[2] of 14 typical achromats it was shown that 11 of the 14 had bipartite adaptation curves composed of two independent sections, when proper precautions were taken to insure pre-adaptation to a high luminance. Other investigators,[3–5] who have reported such bipartite curves for the achromat, are Wölfflin (one case), Lewis and Mandelbaum (three siblings), and Walls and Heath (two cases, not related).

It appears well established, therefore, that a majority of typical achromats have receptors other than rods. There is, however, no general agreement as to the exact nature of these photopic receptors. Larsen's study suggests that from a structural point of view they resemble cones rather than rods. Our previous study showed, however, that the thresholds of these receptors are from one to two log units higher than those of the normal cone mechanism. Lewis and Mandelbaum thought they might be either (a) cones which are too few in number to provide good acuity or color discrimination or (b) a special type of rod in which visual purple regeneration is more rapid. Walls proposed a theory that they are "blue cones"; that is, those which in the normal eye provide the sensation of blueness.†

PURPOSE OF PRESENT INVESTIGATION

A measurement of the spectral sensitivity curve of these photopic receptors would determine whether, from the point of view of photosensitive pigments, they should be classed as rods, abnormal cones, isolated blue cones, or something else. It is not a simple problem to secure such data. To insure that the photopic receptors alone and

* From the Wilmer Ophthalmological Institute of The Johns Hopkins University and Hospital. This investigation was supported by Grant B-810 from the National Institutes of Neurological Diseases and Blindness. Presented at the meeting of the Eastern Section of the Association for Research in Ophthalmology, Washington, D.C., January 17, 1958.

† The fact that the sensation mediated by the achromat's photopic receptors does not differ qualitatively from that mediated by the rods is not a stumbling block to Walls because it is in accord with his "excess hypothesis."[6]

not the scotopic rods are responding, it is necessary to maintain a high level of light adaptation. The stimuli used to measure the spectral sensitivity curve must moreover be of very high intensity to be perceived by the light adapted eye of the achromat. The present study circumvents these difficulties and provides indirect evidence as to the spectral sensitivity curve by measuring the rate of dark adaptation with both white and blue test lights.

Procedure

A detailed description of the apparatus and procedure is given in a previous paper.[7] The white test light has an energy distribution approximating Illuminant C. The blue light is obtained from the same light source by interposing a Wratten no. 47 gelatine filter. The increase in the threshold produced by this selective filter will depend upon the spectral sensitivity of the responding receptors.

Figure 1 shows the spectral transmittance of the blue filter and the spectral luminosity distribution of Illuminant C; (a) for the standard cone sensitivity curve, (b) for a rod sensitivity curve using Weaver's data[8] and (c) for two possible sensitivity curves of blue cone receptors. One of these is the standard Z function of the colorimetrist,[9] the other is the spectral sensitivity curve of a "blue-cone monochromat" studied by Blackwell.[10,11] It can be seen that the relative intensity of the light transmitted by the blue filter will be least for a normal cone receptor system, greatest for one composed of only blue cones, and intermediate for a rod receptor system.

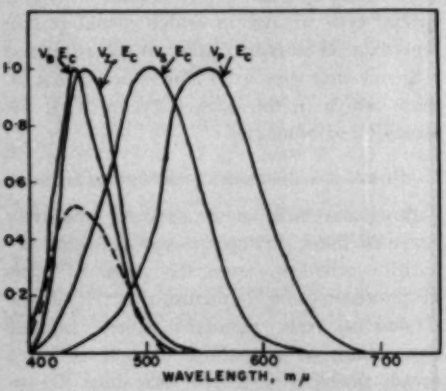

Fig. 1 (Sloan). Basic data for computing proportion of white light (Illuminant C) transmitted by blue filter when receptors have spectral sensitivity of (a) normal cones, (b) normal rods, (c) blue cones. The dashed line shows the spectral transmittance of the blue filter. The other curves show the spectral luminosity distribution of the white light for the different classes of receptor. (See text.)

When the standard cone sensitivity curve is used in the conventional way to compute the total transmittance for Illuminant C, the blue filter is found to have a transmittance of 2.6 percent, that is, a density of 1.6. If the spectral sensitivity curve of the rods is substituted for that of the cones, the computed transmittance is 14.7 percent and the density therefore 0.8. If we assume a sensitivity curve for blue receptors equal to the Z function, the computed transmittance of the blue filter is 41 percent, corresponding to a density of 0.4. The spectral sensitivity curve of one of Blackwell's incomplete achromats gives essentially the same values.

By comparing the experimentally determined density of the blue filter with these computed values it is possible to distinguish between receptors having the spectral sensitivity of normal cones, normal rods, and isolated blue cones. This is illustrated in Figure 2 which shows adaptation curves, for a normal eye, measured with one-degree white and blue test lights at 15 degrees from fixation in the nasal field. In this graph no correction has been made for absorption of light by the blue filter. The uncorrected thresholds for blue light are 1.5 log units higher than those for white in the first section, 0.8 log units higher in the second section. Similar data for a second normal observer showed a separation of 1.3 log units for the first section, 0.8 for the second.

For these two normal observers, therefore, the densities determined experimentally by the second sections of the adaptation curve agree perfectly with the value to be

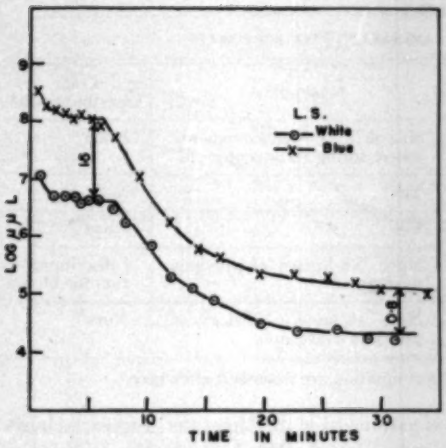

Fig. 2 (Sloan). Paracentral dark adaptation curves (15 degrees nasal) of normal observer for one-degree white and blue test lights. No correction has been made for absorption of light by the blue filter.

expected if these thresholds were determined by rods. The experimentally determined densities for the receptors active during the first stage of dark adaptation, namely 1.5 and 1.3 respectively for the two observers, are slightly less than the computed cone density of 1.6. Several factors might contribute to this difference between experimental and computed values:

1. The experimental data apply to a retinal region 15 degrees from the fovea, whereas the standard photopic sensitivity curve applies to the fovea.

2. Individual sensitivity curves may differ significantly from the standard curve particularly in the short wavelength region of the spectrum.

3. Since the adaptation curves for white and for blue were measured at different times, day-to-day variations in absolute sensitivity could introduce an error in the experimental determination of the density of the blue filter.

Results for achromats

Similar determinations of the adaptation curves for white and blue were made for five achromats. The diagnosis of achromatopsia was based on findings summarized in Table 1. The adaptation curve of the achromat can vary significantly in repeated tests even though the experimental conditions are apparently identical. This variability can be attributed in part to the blinking which usually accompanies exposure to a bright light, making it impossible to reproduce the same level of light adaptation on repetition of the test. The adaptation curves of the achromats for white and blue were therefore determined in the same test session by alternate measurements of the thresholds with and without the blue filter. In four of the five cases the test spot was located 15 degrees from fixation in the nasal field. The fifth subject was tested at the fovea.

Figure 3 shows the adaptation curves of a typical achromat, I. L., and for comparison those of the normal observer whose data are shown in Figure 2. In this graph a correction of 0.8 log unit has been subtracted from the raw thresholds for blue. For the normal subject this brings only the rod sections of the two curves into coincidence. For the achromat *both* sections of the adaptation curves for white and for blue are brought together when a density of 0.8 is assumed for the blue filter. In Fig-

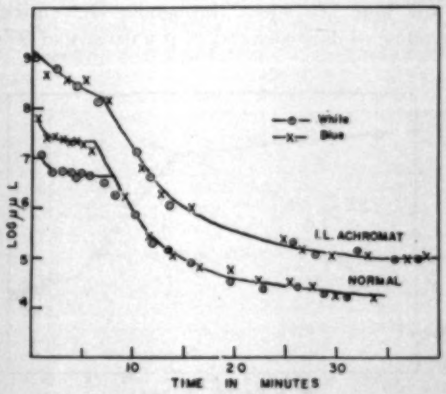

Fig. 3 (Sloan). Paracentral adaptation curves of achromat I. L. and of normal subject. Density of 0.8 assumed for blue filter in this and succeeding graphs.

TABLE 1

RESULTS OF OPHTHALMOLOGIC EXAMINATION. FIVE ACHROMATS

Case	Acuity	Photophobia	Nystagmus	Color Discrimination*
I. L. (F)	14/100	Yes	Not obvious. Fine movements noted during retinoscopy	None
W. Z. (M)	9/100	Yes	Yes	None
J. Z. (M) (Brother of W. Z.)	12/100	Yes	Yes	None
J. D. (M)	20/80	Slight	None. No history of previous nystagmus	? discrimination for blue
V. M. (F)	15/100	Yes	None at present. History of previous nystagmus	None

* The color matching tests and other tests of color discrimination are described elsewhere.[3]

ure 4 the same data for subject I. L. have been plotted with time in logarithmic instead of arithmetic units. This type of plot makes a more obvious distinction between the two different rates of dark adaptation and will therefore be used in presenting the data for the other four achromats.

Figure 5 shows the adaptation curves of the achromat J. Z. Two separate determinations were made of the curves for white and for blue. In the graph the second set of measurements are shifted downward by one log unit to separate them from the first. The first stage of dark adaptation shows a somewhat different rate in the two tests. In each test, however, the thresholds for white and for blue are equal throughout the entire course of dark adaptation, if a density of 0.8 is assumed for the blue filter. Figure 6 shows similar data for W. Z., the brother of the preceding patient. One test showed evidence of slightly higher blue thresholds during the first stage of dark adaptation, indicating that the density of the blue filter is about 1.0 or 1.05 for the first stage, 0.8 for the second. This slight difference was not confirmed in the retest in which both sections indicate a density of 0.8 for the blue filter.

Figure 7 shows two independent determinations of the adaptation curve of the incomplete achromat J. D. As in the previous cases the thresholds for blue and for white lie on the same curve throughout the entire course of dark adaptation when a density of 0.8 is assumed for the blue filter. This patient, as shown by Table 1, has slightly bet-

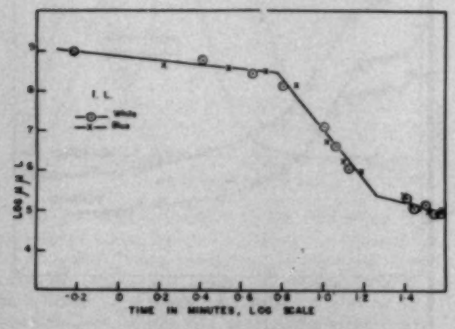

Fig. 4 (Sloan). Data of Figure 3, for subject I. L., plotted with log time as abscissa.

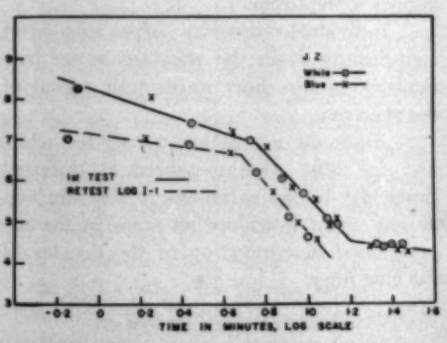

Fig. 5 (Sloan). Paracentral adaptation curves of J. Z.

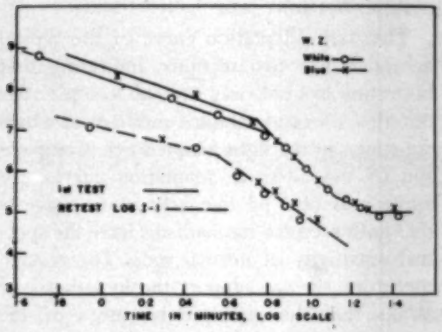

Fig. 6 (Sloan). Paracentral adaptation curves of W. Z.

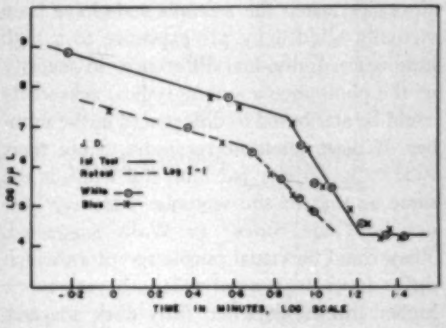

Fig. 7 (Sloan). Paracentral adaptation curves of J. D.

ter acuity than the typical achromat. In color matching tests his inability to obtain a perfect match between blue and white suggests some trace of blue discrimination, at least at the fovea. A few of the achromats of our previous study showed similar evidence of rudimentary color discrimination. Blackwell, who recently studied four incomplete achromats with acuities ranging from 20/50 to 20/100, found evidence for two types of receptor at the fovea, namely rods whose spectral sensitivity was maximal at about 500 mμ and a second type of receptor with maximal sensitivity at about 440 mμ. The adaptation curves of J. D., for a paracentral retinal location 15 degrees from fixation, showed no evidence of such blue sensitive receptors in this region of the retina. Similar tests at the fovea were planned for a second test session, but unfortunately the patient was unable to return.

The foveal adaptation curves of a typical complete achromat are shown in Figure 8. Paracentral fixation controls consisting of two small points of light at three degrees on either side of the one-degree test field were used to aid in its foveal fixation. This subject, V. M.,* a 37-year-old white woman, has outgrown the nystagmus commonly observed in younger achromats. It seems safe to assume, therefore, that foveal fixation of the test field was maintained. Her foveal adaptation curve is bipartite like those of the achromats reported in our previous study.[2] The thresholds for blue and for white are equal throughout the entire course of dark adaptation when a density of 0.8 is assumed for the blue filter. If the foveal receptors responsible for the first section of the curve were "blue cones" with maximal sensitivity of 440 mμ, the blue filter should have a density of only about 0.4.

Comment

If we interpret the bipartite adaptation curve of the complete achromat as evidence for receptors other than normal rods, we can infer that these photopic receptors medi-

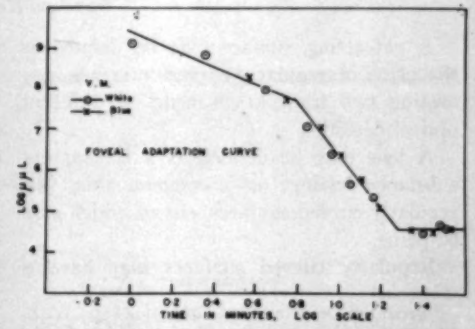

Fig. 8 (Sloan). Foveal adaptation curves of V. M.

* V. M. has also been studied by Goodman and Bornshein[23] primarily by electroretinography.

ate vision when the scotopic rods have been virtually blinded by pre-exposure to a high luminance. Individual differences in severity of the photophobia among typical achromats could be attributed to differences in the number of these photopic receptors. Since their relative sensitivity for blue and white is the same as that of the scotopic rods they cannot be "blue cones" as Walls suggested. They could be visual purple receptors which differ from the normal rods in having much higher thresholds when fully dark adapted, and lower thresholds when they are light adapted.

Summary and Conclusions

The dark adaptation curve of the typical achromat has two sections, indicating that his retina has not only normal scotopic rods but also a second receptor mechanism which functions in the light adapted eye. Comparison of the bipartite adaptation curves for white and blue of five achromats suggests that both receptor mechanisms have the spectral sensitivity of normal rods. The results, therefore, do not support the hypothesis of Walls that the photopic receptors of the achromat are blue cones.

The Johns Hopkins Hospital (5).

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CONOID REFRACTING SURFACES AND CONOID LENSES*

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A refracting surface may be defined as the plane or regularly curved interface separating two transparent medii of different optical densities.

A lens may be defined as a transparent substance having, on a common axis, two regularly curved surfaces, one of which may be plane.

Regularly curved surfaces may have a variety of shapes, but this paper is concerned only with the surfaces of conoids. These surfaces of revolution are obtained by rotating conic sections about their axes (fig. 1). The particular conoids with which this paper deals are the sphere (or circloid) prolate elipsoid, paraboloid, and the hyperboloid of revolution about the transverse axis. These conoid surfaces have been utilized to produce lenses which have a minimum of aberrations.

The conic sections can be obtained by

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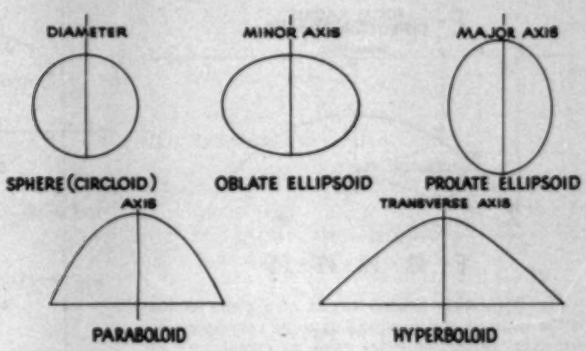

Fig. 1 (Volk). Conoid surfaces are surfaces of revolution obtained by rotating conic sections about their axes.

plane-sectioning a right circular cone (fig. 2). Two basic types of curves result: (1) closed curves, or ellipses, and, (2) open curves or hyperbolas. Separating these two classes of curves is a special case known as the parabola.

History

Kepler in the 17th century was the first to discover that under certain special conditions a hyperbolic refracting surface was free from spherical aberration along the axis. Later Descartes discovered the general equation of surfaces which are free from spherical aberration along the axis, the cartesian optical surfaces. Descartes attempted without success to produce hyperboloidal and other aspherical optical surfaces.†

In relatively recent years aspherical lenses have been successfully made, but on a very limited basis. The ease of accurately producing spherical and toric surfaces, and the difficulty in producing aspherical refracting surfaces has consequently greatly limited the science of optics to the study of the properties and aberrations of spherical and toric lenses and combinations of these lenses.

Specifying a conic section (focus and eccentricity)

Conic sections can be described in relation to a point called the focus and a line called the directrix (fig. 3). A line through the focus, perpendicular to the directrix, is the axis of the conic section. Revolving the conic section about the axis produces the conoid surface; the conic section is thus a meridian section of the conoid surface.

The distance from the focus to the apex of the conic section (through which the axis passes) is the axial focal radius, and the distance from the apex of the conic section to the directrix is the directrix distance. The ratio of the axial focal radius to the directrix distance is known as the eccentricity of the conic section. For any point on the conic section, the ratio of the distance from the focus to the distance to the directrix is constant and is also the eccentricity. If the focus and directrix lie on opposite sides of the conic section, eccentricities are positive, and if on the same

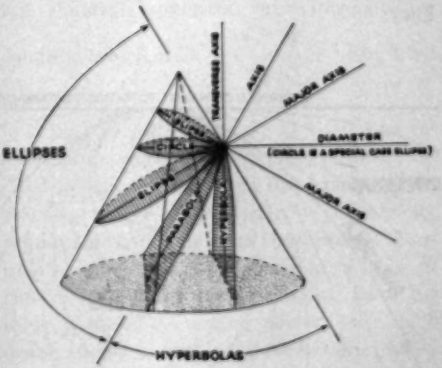

Fig. 2 (Volk). Conic sections are obtained by plane-sectioning a right circular cone.

† Southall, J. P. C.: Mirrors, Prisms and Lenses. New York, Macmillan, 1933.

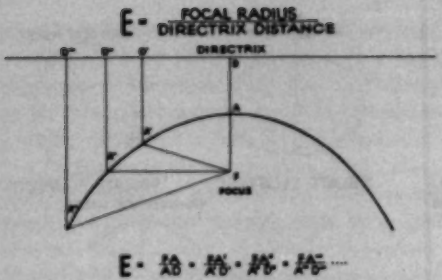

Fig. 3 (Volk). Eccentricity E of a conic section is the ratio of any focal radius to its corresponding directrix distance. Circles have an eccentricity of zero. Ellipses have an eccentricity from zero to one. Parabolas have an eccentricity of one. Hyperbolas have an eccentricity from one to infinity.

side, negative. For the purpose of this paper, only positive eccentricities need be considered.

Eccentricities from zero to one represent ellipses (fig. 4). At zero eccentricity the ellipse is a circle and at eccentricity one the conic section is a parabola. From one to infinity, the conic sections are hyperbolas.

The paraxial portion of all conoid surfaces (the sphere excepted) can be considered approximately spherical, and for this reason the power of a conoid refracting surface can be designated in terms of its paraxial refracting power. The derivation of the equation expressing the paraxial refracting power of conoid refracting surfaces follows:

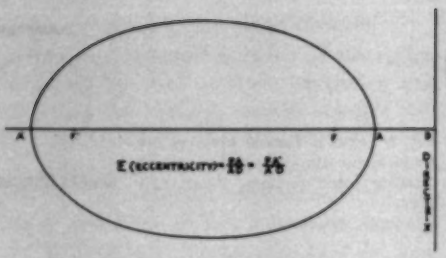

Fig. 5 (Volk). An ellipse. The two foci are symmetrically positioned.

Paraxial refracting power of a conoid surface

(1) From Figure 5, $A'D - AD = AA'$

(2) $$A'D = \frac{FA'}{E}, \quad \text{and} \quad AD = \frac{FA}{E}$$

(3) Substituting from (2) into (1)

$$\frac{FA'}{E} - \frac{FA}{E} = AA', \quad \text{or} \quad \frac{FA' - FA}{E} = AA$$

But $FA' - FA = FF'$

(4) $$\therefore \frac{FF'}{E} = AA', \quad \text{or} \quad E = \frac{FF'}{AA'}$$

(5) From Figure 5

$$AA' = FA + FF' + F'A' = 2FA + FF'$$

(since $FA = F'A'$)

(6) Substituting the value for AA' from (5) into (4)

$$E = \frac{FF'}{2FA + FF'}, \quad \text{or} \quad FF' = 2EFA + EFF$$

(7) But $FF' = F'A - FA$

$$\therefore F'A - FA = EFA + EFA + EF'A - EFA$$

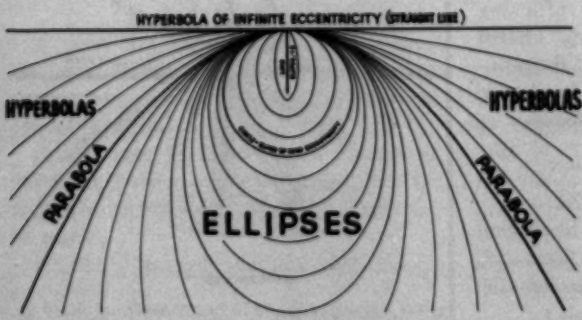

Fig. 4 (Volk). Conic sections viewed as flat surfaces. The axial focal radius for this group is constant while eccentricity is varied.

which reduces to

$$F'A = EFA + EF'A + FA$$

(8) Dividing both sides of the equation in (8) by FA

$$\frac{F'A}{FA} = E + \frac{EF'A}{FA} + 1$$

Transposing

$$\frac{F'A}{FA} - \frac{EF'A}{FA} = E + 1, \text{ or } \frac{F'A}{FA}(1 - E) = E + 1$$

or

$$\frac{F'A}{FA} = \frac{1 + E}{1 - E}$$

(9) Since $\frac{F'A}{FA} = \frac{1 + E}{1 - E}$

Distance F'A may be expressed as 1 + E and distance FA as 1 − E

(10) In Figure 6

$$\angle AFB = \theta$$

$$\therefore \angle AF'B = \frac{(1 - E)}{(1 + E)}\theta$$

(when θ is small, size of angle is inversely proportional to the distance from the side opposite the angle)

(11) $$\angle F'BF = 180 - (180 - \theta) - \frac{(1 - E)}{(1 + E)}\theta$$

$$= \theta - \frac{(1 - E)}{(1 + E)}\theta = \frac{(1 + E)\theta - (1 - E)\theta}{1 + E}$$

$$= \frac{\theta + E\theta - \theta + E\theta}{1 - E}$$

$$= \frac{2E\theta}{1 + E}$$

(12) By construction

$$\angle RBF = \frac{\angle F'BF}{2} = \frac{E\theta}{1 + E}$$

(13) $$\therefore \angle ARB = 180 - (180 - \theta) - \frac{E\theta}{1 + E}$$

$$= \theta - \frac{E\theta}{1 + E} = \frac{(1 + E)\theta - E\theta}{1 + E}$$

$$= \frac{\theta + E\theta - E\theta}{1 + E} = \frac{\theta}{1 + E}$$

(14) When θ is small

$$\frac{RA}{FA} = \frac{\frac{\theta}{\theta}}{1 + E}, \text{ or } RA = FA(1 + E)$$

Let RA = r, the radius of curvature of the paraxial portion of the conic section and FA = f, the axial focal radius of the conic section, then r = f(1 + E), or r = f + EF

(15) Substituting the above value for r in the equation for the power of a spherical refracting surface the new equation is then

D (paraxial refracting power of a conoid surface)

$$= \frac{n' - n}{f(1 + E)}$$

which is the equation for paraxial refracting power of all conoid surfaces, including spheres.

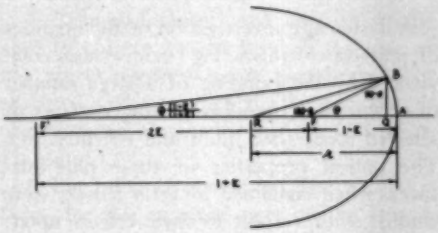

Fig. 6 (Volk). A portion of a conic section in which relative axial distances are expressed in terms of eccentricity. Line RB bisects ∠ F'BF and is perpendicular to the tangent to the conic at point B. Line RB therefore represents the radius of curvature of the paraxial portion of the conic section.

When θ is small, QA may be disregarded and FQ be considered equivalent to FA. Also, angles may be substituted for their tangents.

A characteristic of all conic sections is that if considered as a principal section of a mirror, the two foci are homocentric. Therefore a line bisecting the angle formed by a pair of homocentric rays is normal to the conic section.

In the case of a spherical refracting surface in which eccentricity is zero, the equation for the refracting power is the same as the standard spherical refracting surface equation which is $D = \frac{n' - n}{r}$, r being used instead of f.

With increasing eccentricity there is a reduction in paraxial refracting power (fig. 7). In order to maintain a fixed paraxial refracting power, any change in eccentricity requires a change in axial focal radius. Thus it is possible to make an infinite number of conoid refraction surfaces which have the same paraxial refracting power, but which differ in the refraction peripherally. Away from the apex of the conoid surface (sphere

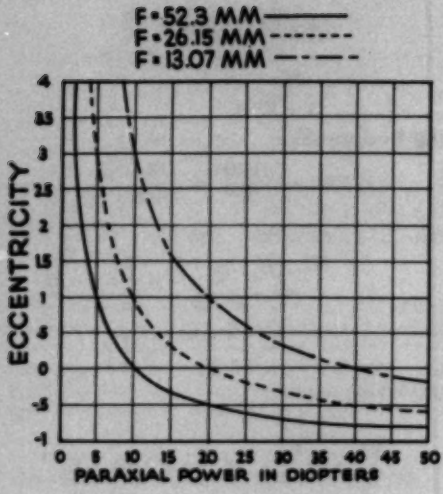

Fig. 7 (Volk). The three curves show, for three different axial focal radii, the change in paraxial refracting power of crown glass conoid surfaces as the eccentricity is varied.

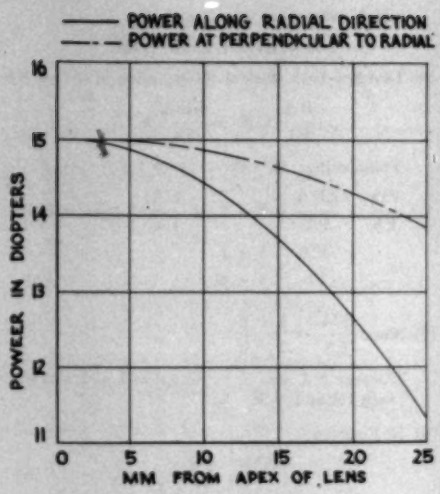

Fig. 9 (Volk). Change in refracting power of a 15-diopter conoid crown glass refracting surface. From the apex peripherally, the surface decreases in power and becomes astigmatic.

excepted), the surface becomes increasingly astigmatic as the periphery of the lens is approached (figs. 8 and 9). The shorter the axial focal radius and the higher the eccentricity, the more rapidly does the surface become astigmatic. Conversely, with relatively long axial focal radii, and moderate eccentricities, there is a relatively larger area of the surface surrounding the apex which is essentially spherical. Consequently, low powered spherical lenses are quite satisfactory for ophthalmic purposes, whereas high powered lenses should be aspheric.

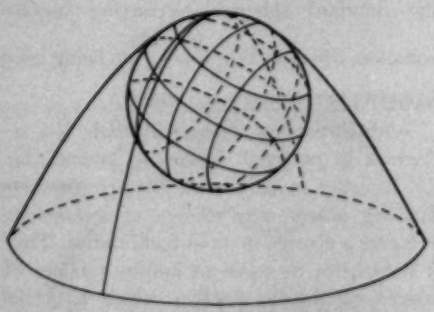

Fig. 8 (Volk). The paraxial portion of a conoid surface (sphere excepted) approximates a spherical surface. With increasing distance from the apex the surface becomes increasingly astigmatic and decreases in power.

Eccentricities and aberrations

A systematic investigation of the qualities of plus conoid refracting surfaces was commenced by the grinding of a large number of crown glass conoid refracting surfaces of selected axial focal radii and eccentricities. The optical properties of these plus surfaces, when combined to form lenses, were studied with respect to their effects upon: (1) Curvature of the field, (2) marginal astigmatism, (3) distortion, (4) relative focal distance for central and peripheral portions of the lens for an axial object point, and (5) chromatic aberration.

From the observations, the following generalizations can be stated with regard to plus conoid lenses.

Insufficient eccentricity results in:

1. Positive curvature of the field. That is, the lens is too strong peripherally, requiring that the object plane be curved about the lens (fig. 10-A).

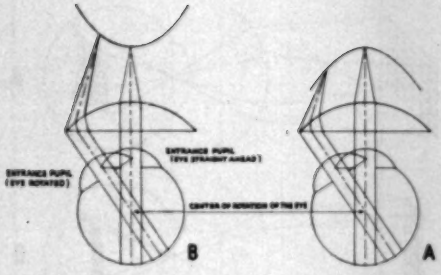

Fig. 10 (Volk). (A) Positive curvature of the field. With insufficient eccentricity it is necessary that the object field be curved about the lens in order to obtain relatively clear vision for all directions of gaze.

(B) Negative curvature of the field. With excessive eccentricity it is necessary that the object field be curved away from the lens in order to obtain relatively clear vision for all directions of gaze.

With the proper amount of eccentricity a flat object field can be seen clearly for all directions of gaze.

2. Positive marginal astigmatism. For an off-axis object point in the primary focal plane, focal lines are produced rather than a stigmatic bundle of rays. The most distal focal line is parallel to the plane of a meridian section in the lens. The focal line closest to the lens is at right angles (fig. 11-A).

3. "Pin-cushioning" and concave apearance to objects viewed through the lens. A flat object appears in its periphery to be enlarged and closer to the viewer who is observing an object through the lens (figs. 12-A and 12-B).

4. Lateral over-correction. The focal distance for the paraxial part of the lens is longer than the approximate focal distance for the periphery of the lens (fig. 13-A).

5. Increased chromatic aberration. (The colored blur is incorporated into and masked by the other aberrations.) (fig. 14-A).

Excessive eccentricity results in:

1. Negative curvature of the field. The object plane is curved away from the lens (fig. 10-B).

2. Negative marginal astigmatism. For an off-axis object at a distance sufficiently greater than the primary focal plane, the focal line closest to the lens is parallel to a

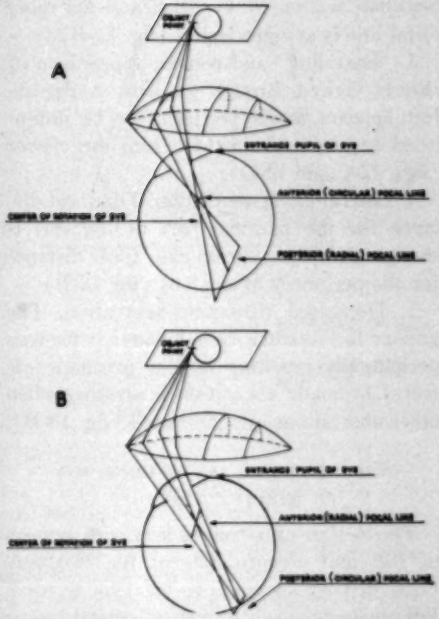

Fig. 11 (Volk). (A) Positive marginal astigmatism. With insufficient eccentricity, the light rays directed toward the lateral portion of the entrance pupil of the rotated eye are refracted proportionately more than those rays directed toward the medial portion of the entrance pupil. Consequently a type of astigmatism is produced in which a homocentric bundle of light rays from an off-axis point in the object plane are refracted such as to concentrate in two "focal lines." The posterior focal line lies in the meridian section of the lens which passes through the approximate center of the entrance pupil. This type of focal line may be called "radial" since a radial group of object points lying in the object plane will form a series of focal lines which overlap in a radial direction.

The anterior focal line lies in a plane perpendicular to the above mentioned meridian section. This type of focal line may be called "circular" since a series of focal lines from a circle of object points lying in the object plane and concentric to the axis of the lens will overlap to form a circle.

(B) Negative marginal astigmatism. With excessive eccentricity the light rays directed toward the lateral portion of the entrance pupil are refracted proportionately less than those rays directed toward the medial portion of the entrance pupil. The astigmatism produced results in a reversal of the position of the radial and circular focal lines as found in insufficient eccentricity.

With the proper eccentricity the bundle of light rays directed toward the entrance pupil will be homocentric for the various positions of rotation of the eye behind the lens.

meridian section of the lens, and the more distal line is at right angles (fig. 11-B).

3. "Barreling" and convex appearance of objects viewed through the lens. A flat object appears in its periphery to be diminished in size and further from the viewer (figs. 12-C and 12-D).

4. Lateral under-correction. The focal distance for the paraxial part of the lens is shorter than the approximate focal distance for the periphery of the lens (fig. 13-B).

5. Decreased chromatic aberration. The greater the eccentricity, the flatter is the lens peripherally resulting in less prismatic effect. Chromatic aberration is striking when other aberrations are eliminated (fig. 14-B).

Conoid lenses are designed for specific tasks

The design of a conoid lens with respect to foci and eccentricities of the two surfaces depends upon how the lens is used. For example, the first set of conoid lenses made were for use as strong plus spectacle lenses for reading in patients with markedly reduced vision. They were designed to be worn 23 mm. from the center of rotation of the eye. The reading material was to be held at the primary focal plane of the lens. The lens was to be centered directly in front of the eye with the optic axis of the lens coinciding with the line of sight of the eye. The diameter of the lens was to be 40 mm. All aberrations were to be corrected for an eye which rotated behind the lens. It was further desired that insofar as possible, the lens should be designed with plano or spherical surfaces on the eye side, so as to make possible the incorporation of sphere and cylinder into the prescribed lens (fig. 15).

If designed in the symmetric biconvex form, focus and eccentricity of the surfaces of crown glass conoid lenses for reduced vision would be plotted along a curve as shown in Figure 16.

Symmetric biconvex conoid lenses are of excellent quality with respect to correction of aberrations, except for slight distortion

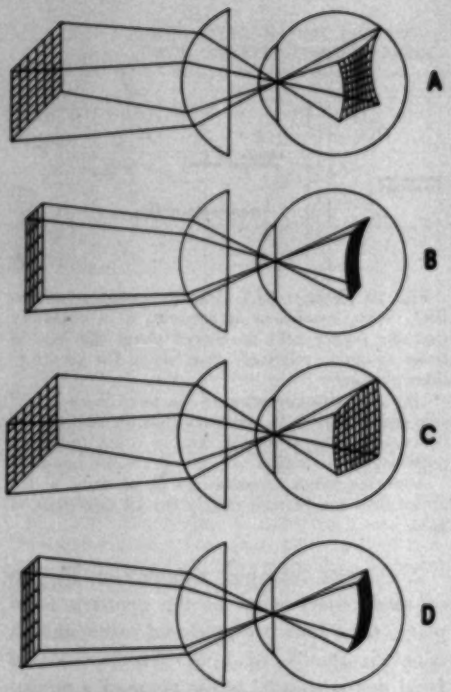

Fig. 12 (Volk). Distortion. (A) "Pin-cushioning" and (B) "concaving" of the image. Insufficient eccentricity results in an increasing amount of magnification of objects seen through peripheral portions of the lens. A geometric rate of increase in magnification of the image through increasingly peripheral parts of the lens results in a flat square object developing a "tufted or pin-cushioned" appearance, as well as appearing to be hollowed or "concaved." A lateral or vertical movement of the object in its focal plane results in it appearing to roll around the lens.

(C) "Barreling" and (D) "convexing" of the image. Excessive eccentricity results in decreasing amounts of magnification of objects seen through peripheral portions of the lens. A geometric rate of decrease in magnification of the image through increasingly peripheral parts of the lens results in a flat square object appearing "barreled" as well as appearing to be curved away from the lens or "convexed."

A lateral or vertical movement of the object in its focal plane results in its appearing to roll away from the lens.

in which objects viewed through the lens appear slightly concave and enlarged peripherally. This slight distortion can be eliminated while maintaining the correction of

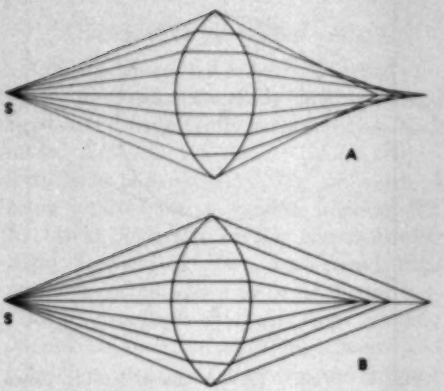

Fig. 13 (Volk). (A) Lateral over-correction. With insufficient eccentricity, light from an axial object point-source, S, which lies at a distance from the lens greater than the anterior focal distance, is refracted by the lens such that light rays from peripheral zones of the lens cross the axis closer to the lens than paraxial rays. If the lens has spherical surfaces (one may be plane) the aberration is known as spherical aberration.

(B) Lateral under-correction. With excessive eccentricity, light from an axial object point-source, S, which lies at a sufficiently greater distance from the lens than the anterior focal distance, is refracted such that light rays coming from peripheral zones of the lens cross the axis at a greater distance from the lens than paraxial rays.

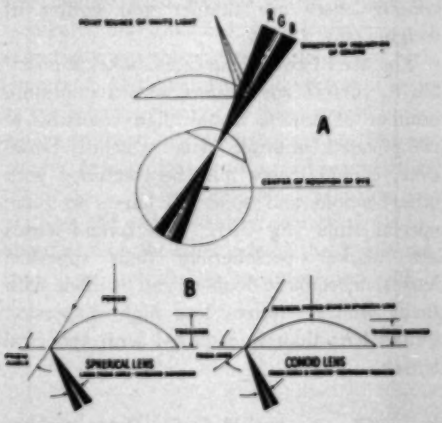

Fig. 14 (Volk). (A) Schematic representation of chromatic aberration produced by a strong plus lens in front of the eye. The more highly refracted "blue" light B must pass through a more peripheral part of the lens in order to enter the entrance pupil of the eye. The subjective impression of a point source of white light is a rainbow-like blur in a radial direction with the red R seen most centrally and the blue seen most peripherally.

(B) A comparison of chromatic aberration in a spherical lens and a conoid lens. The spherical lens has insufficient eccentricity and consequently more prismatic effect laterally. The conoid lens with its increased eccentricity flattens peripherally and has less prismatic effect. In the spherical lens chromatic aberration is not very apparent since it becomes part of a general blur. In the conoid lens in which other blurring aberrations have been eliminated, chromatic aberration can be readily seen.

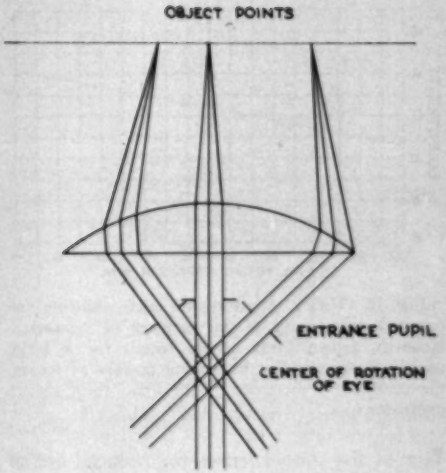

Fig. 15 (Volk). Schematic representation of a conoid lens in front of the rotating eye. For each position of the eye a homocentric bundle of light rays proceeds from a point in the object plane toward the conoid lens and, after refraction by the lens, proceeds toward the entrance pupil of the eye as a homocentric bundle of light rays. In the case depicted here, the object plane coincides with the primary focal plane of the lens and light rays are parallel in each homocentric bundle of rays proceeding toward the entrance pupil for all positions of the eye.

the other aberrations by making the eccentricites and paraxial powers of the two surfaces different. Decreasing the eccentricity of one surface while maintaining paraxial power of each of the two surfaces of the lens necessitates an increase in eccentricity in the other. The use of plane and spherical surfaces (zero eccentricity) as the back sur-

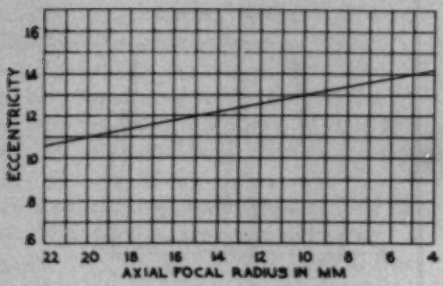

Fig. 16 (Volk). Approximate curve showing focus and eccentricity of the surfaces of symmetric biconvex crown glass conoid lenses for a large range of powers. For increasing powers of lenses, the eccentricity increases as the axial focal radius decreases.

face of the conoid lenses for reduced vision requires a relatively high power and high eccentricity on the front surface. Thus, the first seven conoid lenses of the series of 10 lenses for reduced vision not only are higher in power on the front surface but also show a rather large increase in eccentricity as the power of the back spherical surface increases.

The last three lenses of the series have been made symmetric biconvex conoids, disregarding the slight distortion which results. It was felt that it would be less confusing to opticians if they did not have to identify front and back aspheric surfaces, both of which would be highly curved and similar in appearance.

Additional advantages of conoid lenses

As a result of the high eccentricities utilized in conoid lenses, there is considerable flattening of the lenses peripherally, making it possible to produce lenses of large diameter, even in the higher powers where such diameters are impossible with spherical

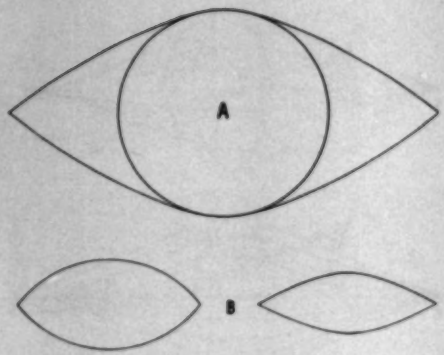

Fig. 17 (Volk). (A) A conoid lens is shown enclosing a spherical lens whose thickness and power are identical to the paraxial thickness and power of the conoid lens. Since a conoid lens flattens peripherally, it is apparent that its diameter can always be greater than that of the sphere. (B) A symmetric biconvex spherical lens is compared to a symmetric biconvex conoid lens of equivalent paraxial power. The considerable reduction in thickness of the conoid lens is accomplished by both a reduced paraxial curvature as compared to the spherical lens and a peripheral flattening of the surfaces.

surfaces (fig. 17-A). Where the same diameters are obtainable in conoid and spherical lenses of equivalent paraxial power, the conoid lenses are thinner and lighter in weight (fig. 17-B).

The use of conoid surfaces makes it possible to correct aberrations with a minimum number of lenses, rather than resorting to complicated multiple lens systems. However, conoid lenses may be combined with other conoid and spherical lenses to form special units (fig. 18). Since conoid lenses are weaker peripherally than spherical lenses, achromatic doublets can be made with them which requires less high dispersion minus lens than is required with spherical lenses.

Fig. 18 (Volk). Some combinations using conoid lenses. Extremely high magnification semi-achromatic conoid doublets have been produced, which have a relatively wide clear field.

Other uses of conoid lenses

Conoid lenses have been designed for other purposes. Excellent high powered hand-held magnifying lenses have been made. Aspheric achromatic camera objectives have been designed, one of which is being utilized in a fundus camera. Dr. R. David Sudarsky of the Eye-Bank for Sight Restoration, Inc., has found high powered conoid lenses to be of distinct advantage in indirect ophthalmoscopy, making possible the use of much stronger lenses and providing increased clearness and size of the field. This aspect of the use of conoid lenses is to be reported in a separate paper. A project involving the design and production of conoid cataract bifocals is now in progress.

Future possibilities

The concept of lenses of varying power should not be limited to the conoid lenses just described. Other types of regularly-curved nonspherical surfaces will certainly find a use in ophthalmology and related optical fields. The hyperbolic paraboloid (fig. 19), is one such surface which I have investigated and analyzed. In low powers it is essentially equivalent to a saddle-shaped toric surface. Such a surface could very well serve as the inner surface of present conoid lenses which are used in subnormal vision. For example: In a patient with mixed astigmatism requiring a 20-diopter conoid addition for reading, the saddle-shaped correction should be ground on the back side of the 20-diopter conoid lens which has been designed with a

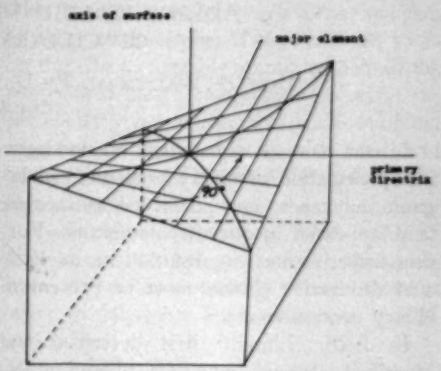

Fig. 19 (Volk). Rectangular hyperbolic paraboloid. The surface is known as a ruled warped surface.

20-diopter paraxial power ellipsoid as the front surface. Conoid cataract lenses may likewise utilize a saddle-shaped toric inner surface.

In presbyopia, lenses which vary in power may have an advantage over bifocals for certain types of work.

Condensing lenses for projectors can be produced which are more efficient in light gathering and which will give a more uniform brightness for its various zones.

Conoid lenses can be used as the objective of Galilean telescopic spectacle lenses, enabling a wider and clearer field. They may also be used as the eyepiece of astronomical and terrestrial telescopes. For both of these purposes, they may be combined with high dispersion minus lens to make an achromatic doublet.

2460 Fairmount Boulevard (6).

ADENYLIC ACID DEAMINASE ACTIVITY OF CILIARY PROCESSES*

MORTON B. WAITZMAN, PH.D., AND ELMER J. BALLINTINE, M.D.
Cleveland, Ohio

In the course of studies on oxidative phosphorylation of ciliary processes, an organic substance, now identified as inosinic acid, appeared in our chromatograms. Further experiments revealed that an adenylic acid deaminase system must be present in ciliary process tissue.

In 1928, Schmidt[1] first described and identified, through ammonia liberation experiments, a specific 5-AMP† deaminase in muscle. We still know very little about the mechanism of action and the precise physiologic role of this enzyme. Many efforts have been made to identify further AMP deaminase. For example, Kalckar, in 1947,[2] presented methods for a spectrophotometric identification; Kutscher, in 1948,[3] described relative activity of the enzyme in different species and tissues; and in 1957, Ya-Pin Lee[4-6] in a series of papers reported isolation and chemical characteristics of a crystalline form of the enzyme in rabbit skeletal muscle. With the exception of an adenylic acid deaminase of dog brain[7] and one from ox brain,[8] these other deaminases have shown no activity potentiation in the presence of ATP or ADP.

METHODS

Tissue. Three different tissues (hog, rabbit albino, and rabbit pigmented processes) were used. Eyes were enucleated immediately after slaughter and transported to the laboratory in iced containers.

Experimental. The ciliary processes were dissected, homogenized in appropriate salt and buffer media, and aliquots were taken to make a five-percent tissue suspension in the incubation media.

Acetone powder preparations were made by extracting tissue three times in dry ice temperature acetone, lyophilizing the particles to dryness over a high vacuum pump, homogenizing the particles with ice water in Potter-Elvehjem glass homogenizers, centrifuging, and incubating the supernatant containing the soluble enzyme.

All Warburg vessel reactions were under an atmosphere of 95 percent N_2 — 5.0 percent CO_2 and were stopped by adjusting the incubation media to a final concentration of 3.0 percent TCA. The supernatant was then analyzed for the soluble nucleotide fractions.

Analytic procedures for organic and inorganic P containing compounds have been described previously.[9] Ammonia release was measured by the Conway cup incubation (room air environment) methods.[10]

Special chromatographic methods. Methods of chromatography used to isolate the various nucleotide, nucleoside, and inorganic P components have been reported previously.[9]

RESULTS

A typical chromatographic separation, showing relative phosphorus content of the molecules, is seen in Figure 1. The short strip contains P spots, and in order away from P are ITP, IDP, IMP, ATP, ADP, and AMP. IMP and AMP are not too visible because of their low free-phosphorus content. The number and type of P-containing compounds sought are shown on the schematic diagram of Figure 2.

* From Western Reserve University, School of Medicine, and the University Hospitals of Cleveland. This study was supported in part by Research Grant B-121 from the National Institute of Neurological Diseases and Blindness, National Institutes of Health.

† The following abbreviations are used: ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; ITP, inosine triphosphate; IDP, inosine diphosphate; IMP, inosine monophosphate; P, orthophosphate; and TCA, trichloroacetic acid.

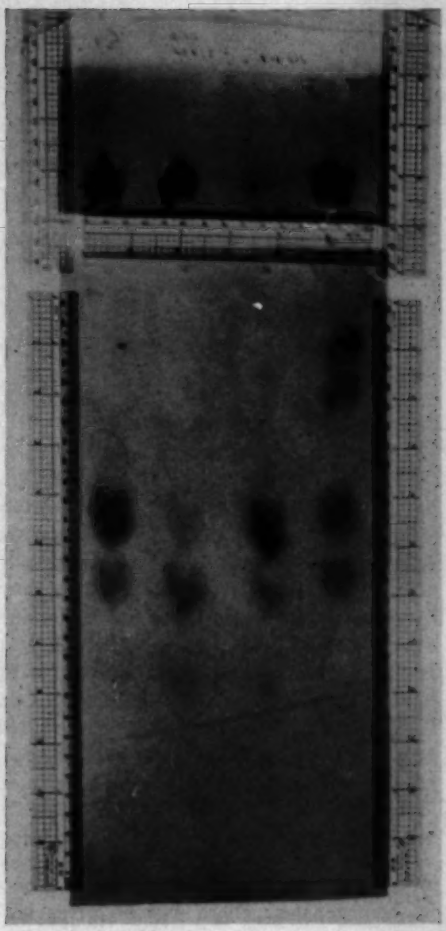

Fig. 1 (Waitzman and Ballintine). Chromatographic separation of adenine and inosine nucleotides.

The IMP on the chromatograms has been identified by observing adjacent migration of standard IMP spots, and by spectrophotometric absorption of narrow paper strips containing the nucleotide spots. A typical spectral analysis, demonstrating the expected peaks for the various nucleotides, is shown in Figure 3.

Balance studies

Phosphorus. In all experiments supernatant phosphorus analyses were compared with chromatography phosphorus analyses in order to determine if the observed spots on the chromatograms accounted for all the phosphorus-bound compounds. In some experiments seven-minute acid labile P of the supernatant was compared with total acid labile P in the ATP and ADP spots of the chromatograms.

Nucleotide. Comparisons were made of total chromatogram nucleotide content between the different periods of incubation in order to determine accumulation or loss of nucleotide.

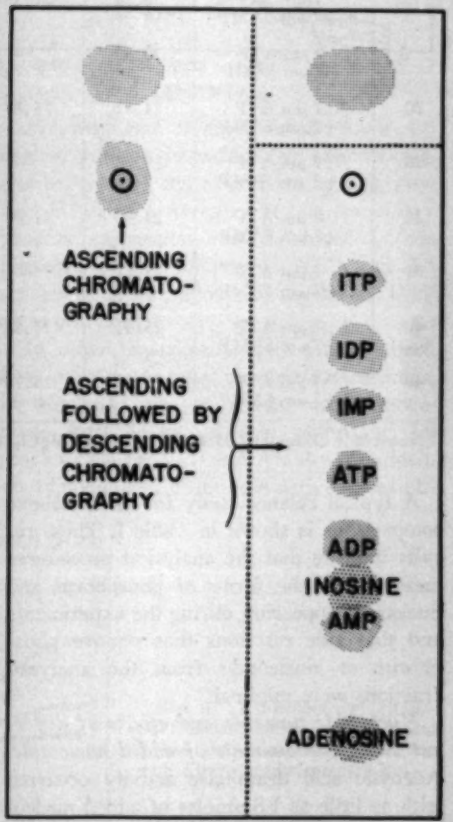

Fig. 2 (Waitzman and Ballintine). Schematic diagram of relative chromatographic migration rates of inosine and adenosine and their derivatives. Ascending solvent, isopropyl ether and formic acid; descending solvent, isobutyric acid, ammonium hydroxide, and Versene.

TABLE 1

FRESH HOG EYE PREPARATION

(Tris-succinate buffer, pH 7.4)

A. SUPERNATANT

Time	Content	Supernatant P (A)	Total P Chromatogram Fractions (B)	(B/A)	Total Nucleotide Content on Chromatogram	7 Min. Acid Labile Supernatant P	Labile P Chromatogram Nucleotide Fractions
(min.)		μmoles	μmoles		μmoles	μmoles	μmoles
0	7 μm ATP +7 μm 5AMP	28.14	27.09	0.96	13.46	—	13.63
0	7 μm ATP +7 μm 5AMP	31.08	28.14	0.90	14.46	13.65	13.68
5	7 μm ATP +7 μm 5AMP	28.14	27.35	0.97	14.00	13.23	13.35
5	7 μm ATP +7 μm 5AMP	30.66	—	—	—	13.02	—
20	7 μm ATP +7 μm 5AMP	24.57	25.83	1.05	13.10	12.18	12.73
20	7 μm ATP +7 μm 5AMP	28.98	—	—	—	12.18	—
30	7 μm ATP +7 μm 5AMP	27.72	24.08	0.87	12.25	10.08	11.83
30	7 μm ATP +7 μm 5AMP	25.41	—	—	—	10.08	—
40	7 μm ATP +7 μm 5AMP	25.41	27.83	1.10	14.30	14.70	13.53
40	7 μm ATP +7 μm 5AMP	31.50	28.78	0.91	14.72	13.23	14.06

Note: Salt Content: 0.05 M KCl; 0.05 M $MgCl_2$; 0.11 M NaCl.

A typical balance study for all the above comparisons is shown in Table 1. These results indicate that the analytical procedures measured all the forms of phosphorus and nucleotide appearing during the experiments and that side reactions that remove phosphorus or nucleotide from the analyzed fractions were minimal.

Nucleotide turnover and effects of different kinds and amounts of added nucleotide. Adenylic acid deaminase activity occurred with as little as 1.8 μmoles of added nucleotide and was most marked when AMP plus either ATP or ADP was added in equimolar amounts. Figure 4 shows the results of an experiment in which 7.0 μmoles each of ATP and 5-AMP were incubated with the supernatant from fresh hog eye ciliary process homogenates. There was no great net loss or gain of total nucleotides during the incubations. Experiments with standard spots indicated that less than 0.25 γ of purine could not be detected with ultraviolet light. This could account for 0.25 μmole of adenosine in the supernatant which would be really undetected.

pH effects. A pH optimum with $-KPO_4$ buffer was demonstrated (fig. 5). A broad peak is seen at about 7.0 with high activity from 6.0 to 8.0. Similar optima were demonstrated also for stored residue and whole tissue and acetone powder preparations of hog eyes.

Inosinic acid accumulation vs. NH_3 pro-

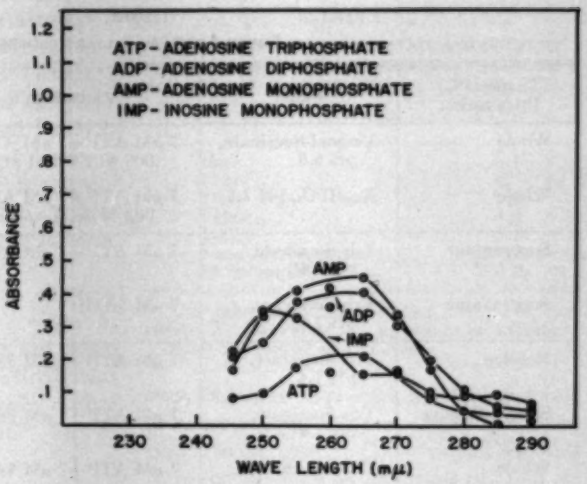

Fig. 3 (Waitzman and Ballintine). Spectral analysis of paper strip cut through nucleotide-containing spots on chromatogram.

duction. Comparative studies between the ammonia release and inosinic acid accumulation under ideal substrate and pH conditions were made. It is expected that under similar conditions these measurements should obey a 1:1 stoichiometry. This ratio was approached, in fact, as shown in Table 2. It should be noted that the Conway cup incubations were at slightly higher temperatures (about 26°C. compared with 20°C.) and that the Conway cups were incubated about 10 minutes longer in the tissue fraction experiment. This would account, in part, for some of the discrepancies.

Freezing and storage: whole tissue vs. acetone powder. In each of two experiments, one before and one after freezing and storage, in which a five-percent hog eye ciliary process suspension was incubated in the presence of 3.5 μmoles of ATP and 3.5 μmoles of AMP, about 2.0 μmoles of IMP were produced.

In other experiments with frozen-stored hog process acetone powder preparations, in which 0.5 gm. of powder was homogenized with 10 ml. of cold water and 7.0 μmoles each of ATP and AMP were added, no adenylic acid deaminase activity was ob-

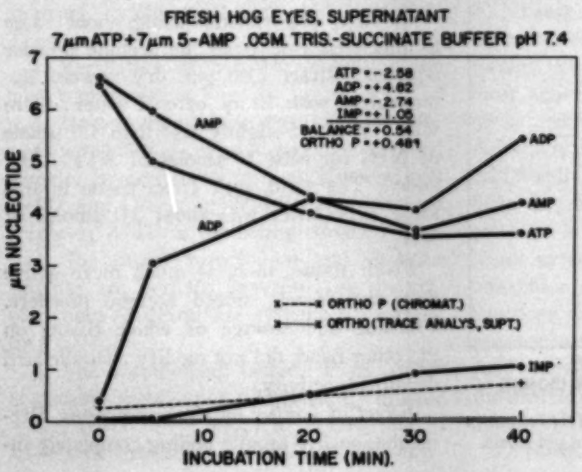

Fig. 4 (Waitzman and Ballintine). Balance study, nucleotide and P loss and gain during incubation with ciliary process preparation.

TABLE 2

FRESH HOG EYES' CILIARY PROCESSES

Tissue (5%) Preparation	Buffer	Substrate	μM IMP Evolved[1]	[1]μM NH_3 Evolved[2]
Whole	Veronal-Succinate, pH 6.8	7 μM ATP+7 μM AMP .003 M EDTA; Mg. Free	.14[3]; 2.31	2.04
Whole	Na_2HPO_4, pH 7.4	7 μM ATP+7 μM AMP .003 M EDTA; Mg. Free	2.70	2.21
Supernatant	Tris-Succinate, pH 7.4	7 μM ATP+7 μM 5AMP	1.05	1.29
Supernatant	Tris-Succinate, pH 7.4	7 μM 5AMP	.26	.53
Residue	Tris-Succinate, pH 7.4	7 μM ATP+7 μM 5AMP	1.68	1.85
Supt.+Residue	Tris-Succinate, pH 7.4	7 μM ATP+7 μM 5AMP	2.31	3.04
Whole	Tris-Succinate, pH 7.4	7 μM ATP+7 μM 5AMP	2.78	3.83

[1] 40 Minute Anaerobic Warburg Incubation.
[2] 40–50 Minute Room Air Conway Cup Incubation.
[3] 40 Minute Room Air Incubation.
Note: Salt Content: .05 M KCl; .05 M $MgCl_2$; .11 M NaCl.

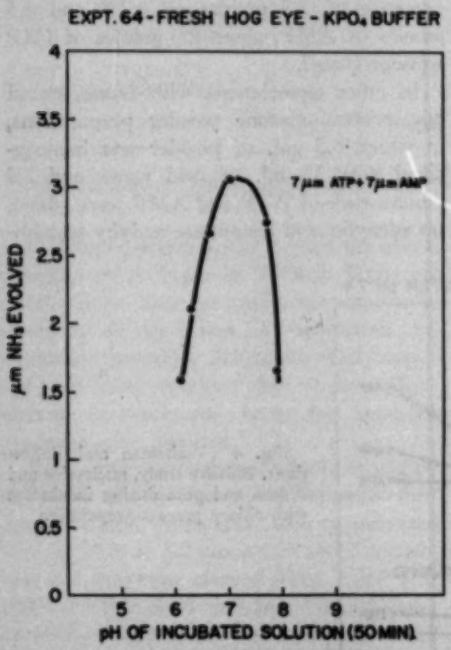

Fig. 5 (Waitzman and Ballintine). NH_3 release in presence of -KPO_4 buffer at different pH levels.

served with the spectrophotometer; that is, there was no change in the absorption pattern at 248.5 mμ. The same type of preparations yielded no inosinic acid accumulation in Warburg vessels, and only slight NH_3 release in Conway cups. A similar preparation using rabbit albino eyes, but with one-half the amount of nucleotides, yielded about 0.70 μmole of NH_3.

In other experiments using about twice as much enzyme from the acetone powder aqueous extract (1.0 gm. dry powder homogenized with 10 ml. of cold water) there was a yield of slightly less than 1.0 μmole of NH_3 for each 14 μmoles of ATP-AMP added. The yield with fresh tissue in this same experiment was about 3.0 μmoles of NH_3.

Fresh tissue, then, is much more active than the frozen, stored acetone powders. Freezing and storage of whole tissue, on the other hand, did not modify adenylic acid deaminase activity.

Effect of boiling on tissue fractions. Pre-incubation five-minute boiling completely in-

activated adenylic acid deaminase activity in granule and supernatant preparations from fresh hog eye ciliary processes incubated for 40 minutes in $-NaPO_4$ buffer (pH 7.4) and 3.5 μmoles each of ATP and 5-AMP. Unboiled preparations in similar media yielded about 1.2 μmoles of IMP for supernatant and 1.1 μmoles for residue. A five-percent whole tissue suspension equivalent was used in both fractions.

Enzyme specificity. Preparations of fresh hog eye (described previously) when 5-AMP + ATP was the substrate and when marked adenylic acid deaminase activity was observed were totally inactive when similar preparations substituting 3-AMP for 5-AMP were employed.

Discussion and Conclusions

The results presented indicate that the ciliary process tissue of the species tested contains a specific 5-adenylic acid deaminase system that is active maximally around neutrality. The question as to whether two AMP deaminases (and not one), one soluble and one insoluble, exist in this tissue must be explored further. Also, the specific roles of ATP and ADP as co-factors must be investigated further.

Practical considerations. The adenylic acid deaminase found in ciliary process might play certain physiologic roles in aqueous humor dynamics and secretion. These are:

1. Regulation of 5-AMP content in the ciliary processes.

2. Help to maintain normal vascular supply by regulating, in part, patency of blood vessels. Investigators such as Kutscher, in 1948,[3] and Green, in 1950,[11] have indicated a role of AMP in producing coronary dilation. Peripheral vasodilation and vascular collapse are perhaps prevented by discrete placement of deaminase systems for regulatory purposes.

3. The metabolism factor: P of AMP is a stable group—not a high energy linkage—and AMP itself plays a limited role in nucleotide exchange reactions. AMP, however, has been implicated as playing a role in many enzyme systems. These would include (as discussed in Colowick and Kaplan's series on *Methods in Enzymology*[12]) an activation of isocitric dehydrogenase, protection of TPN, inhibition of pentose-PO_4-isomerase, involvement in ion pumps, such as sodium pump mechanisms, and so forth. If AMP plays these specific metabolic and functional roles and if AMP is somewhat of a by-product of nucleotide interaction, the importance of the role of adenylic acid deaminase in regulating AMP concentration becomes apparent.

TABLE 3

AMP deaminase activity in cats and chickens*

Organ	1/10 Gespaltet %
Herz	0,8
Milz	1,6
Niere	2,0
Leber	3,0
Lunge	5,5
Skeletmuskel, weiss	100,0
Skeletmuskel, rot	70,0

* As determined by Kutscher, W., and Sarreither, W.[3] (Reprinted with permission of publisher.)
Note: See text for explanation.

It is of interest to compare the ciliary process adenylic acid deaminase with deaminases of other systems. Kutscher[3] reported activity in cats and chickens (table 3). These figures refer to percent AMP deaminated in one hour by 100 mg. of wet tissue. When we put our data, using our most active hog eye process system tested, on a comparative basis with the data of Kutscher, we derived a figure of 86 percent AMP splitting—an activity, certainly, of no little importance. The ciliary process deaminase is potentiated by ATP, and to a lesser extent, by ADP. In this property it resembles the deaminase from particulate fractions of ox brain[8] and from the acetone powder preparations of dog brain,[7] in which ATP is obligatory; it is distinguished from the muscle deaminases[1] which apparently are unaffected by ATP.

Department of Pharmacology.

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VISUAL SENSORY UNITS AND THE MINIMAL ANGLE OF RESOLUTION*

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Los Angeles, California

It is now 100 years since Aubert and Förster demonstrated that visual acuity is better centrally than peripherally and that the decline of sensitivity is gradual and orderly. Although this relation is now generally recognized and is known to apply to other capacities of the retina, the details and the underlying mechanism are far from being satisfactorily explored. The importance of this gradient is well recognized. Polyak, in his monumental work on the retina, gives as the seventh reason for undertaking such a task the fact that the "known structures are inadequate to explain the retinal gradient. Even such an apparently elementary problem as to what anatomical factors are responsible for the striking difference between the central and the peripheral acuity . . . remains unsolved. . . ." (Polyak, 1941, p. 186). It is only necessary to point out the relation of the gradient to such clinical problems as the loss of acuity due to central scotomas, small angle squints, and amblyopia, for example, to show the practical importance of a detailed knowledge of the retinal gradient. Despite interest, the difficulties of the experiments have limited the number of attempts and have prevented wholly satisfactory results.

A contributing cause to the difficulty of analysis has been the absence of any simple mathematical characterization of the curve of acuity as a function of distance from the fovea. The curves are often described as a fall of acuity "at first very rapid but later progressively slower" or some similar expression which could apply with equal vagueness to several distinct mathematical curves. Precision is necessary for effective comparison of curves from different individuals or obtained under different conditions.

Gradient in terms of minimal angle of resolution

It is, therefore, of interest that the minimal angle of resolution, the reciprocal of the visual acuity, presents a much simpler picture of the gradient than does the acuity. The eye is unique in that the sensitivity has been used for its rating rather than the threshold, as is customary with other sense organs, but the minimal angle of resolution (MAR), a true threshold, is now coming into general use. In Figure 1 are presented the visual acuity and the minimal angle of

* From the Los Angeles College of Optometry.

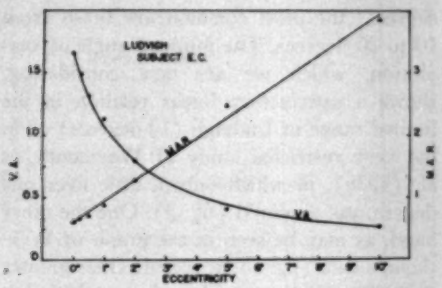

Fig. 1 (Weymouth). Visual acuity and minimal angle of resolution as functions of retinal eccentricity. Data from Ludvigh, subject E. C.

resolution plotted as functions of the distance from the point of fixation in degrees. These values for the light adapted eye are from Ludvigh (1941). It is obvious that these data are reasonably represented by a straight line.

If this relation is close and general enough, a precise and simple description of the curve is available in the constants of the straight line, the slope and intercept. Since the minimal angle of resolution is the reciprocal of the visual acuity, existing data may readily be transformed.

Before generalizing from this example, several questions must be asked. How satisfactory is the fit of the straight line? Does it apply to data from other workers? Does it apply to the full range of eccentricity that has been explored? To turn farther afield: Does the linear relation apply to other thresholds of the eye, and, if so, which ones?

The first question is not easily answered to the satisfaction of the statistician. Published data of this type are traditionally presented in the form of average acuities for

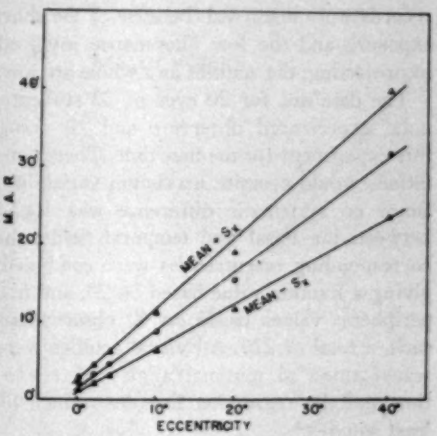

Fig. 2 (Weymouth). Mean minimal angle of resolution and points, one standard deviation above and one standard deviation below, as functions of retinal eccentricity. Data from 20 observers, one eye only.

each eccentricity, and without the original observations none of the measures of reliability can be calculated. The means conceal the variability and in consequence the fit always appears better than it really is. In the absence of the original observations for the classical work, data from a course in physiologic optics have been analyzed; the results are presented in Figure 2 and Table 1.

A shutter was used to limit the time of exposure of a Landolt C at the given eccentricity; this reduced but did not completely eliminate inexact fixation and too high acuities are often obtained, particularly at low eccentricities. On this account and because some observers failed to obtain an acuity within the range of the apparatus at the maximum eccentricity of 20 degrees, a few

TABLE 1

MINIMAL ANGLE OF RESOLUTION BASED ON STUDENT RECORDS

Eccentricity	0°	1°	2°	5°	10°	20°
Number of observations	20	40	40	40	40	40
Minimal angle of resolution (mean)	2.06	3.26	4.58	8.77	15.50	32.08
Standard deviation	0.82	1.17	1.54	2.38	3.71	8.16
Mean + standard deviation	2.88	4.43	6.11	11.15	19.21	40.24
Mean − standard deviation	1.24	2.08	3.04	6.38	11.78	23.93
Standard error of mean	0.18	0.18	0.24	0.38	0.59	1.29

records were discarded. Because of the short exposure and the low illuminance involved in projecting, the acuities as a whole are low.

The data are for 20 eyes of 20 students, none experienced observers and all doing this experiment for the first time. These conditions would promise maximum variability. Since no systematic difference was found between the nasal and temporal fields, the corresponding eccentricities were combined, giving a fixation value based on 20, and five peripheral values based on 40 observations each, a total of 220. All visual acuities were transformed to minimal angles of resolution and the regression line determined by least squares.

The standard deviations for the various eccentricities are obviously not equal, being closely proportional to the angle of resolution, and since the standard error of estimate is not valid for heteroscedastic data, it was not calculated. The graph shows the means with their standard deviations and the fitted regression line. This gives a clear idea of the variability in highly variable data and indicates the validity of the fit of the straight line for this range of eccentricities.

Generality of linear relations

The question of generality is important. The eccentricities explored in the various studies have ranged from about one to 70 degrees; the most common are those from 10 to 20 degrees. The minimal angle of resolution, which we are now considering, shows a satisfactory linear relation in the limited range of Ludvigh (10 degrees) or in the very restricted study of Weymouth, et al. (1928), in which only a little over one degree was explored (fig. 3). One the other hand, as may be seen in the graph of Wertheim's data (fig. 5), at eccentricities greater than 20 or 30 degrees there is a variable but undoubted tendency for the minimal angle of resolution as a function of eccentricity to become greater than expected from lesser eccentricities so that the curve is concave upward.

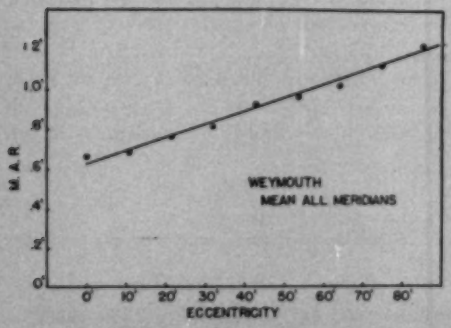

Fig. 3 (Weymouth). Minimal angle of resolution (mean of four meridians) as function of retinal eccentricity. Subject F. F. W.

Several elements may be operative here. The increasingly greater difficulty of the more peripheral observations is notorious; this would result in greater variability but not necessarily in higher values. The true angle of eccentricity presents a problem, since large external (field) angles progressively exceed the corresponding internal angles. Although this error is present and tends to cause the deviation noted, there are no adequate studies permitting correction and the usual field angles have been used.

Light incident from the periphery of the visual field encounters increased reflection and obliquity of the pupil is reduced in intensity (Weale, 1956, p. 393). Accurate values are lacking, but any marked reduction of intensity would require greater angular separation of resolvable detail and would lead to an undue peripheral rise of the minimal angle of resolution.

There are cortical, histologic (Polyak), and perimetric indications (Traquair, 1949, p. 6) of a significant distinction between "central" and "peripheral" with the boundary falling in the neighborhood of 20 or 30 degrees which perhaps would justify treating the two regions separately. In conclusion we may say that for the more important and better known central region the minimal angle of resolution as a function of eccentricity is satisfactorily linear.

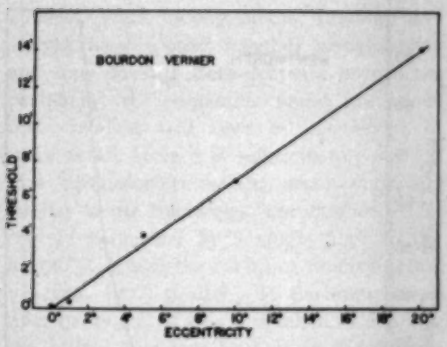

Fig. 4 (Weymouth). Vernier threshold as function of retinal eccentricity. Data from Bourdon.

Types of thresholds showing linear relations

To what types of threshold does the linear relation apply? The minimal angle of resolution of the light adapted eye has been considered; to this may be added the vernier threshold, and several horopteral values. The light threshold of either the light- or the dark-adapted eye is not linear, nor is the critical fusion frequency. Does this incomplete list present any logical basis for classification?

Spatial and intensity thresholds are at once suggested. The visual acuity or the minimal angle of resolution is expressed in spatial terms of angular size, although this is not the only factor involved; this is equally

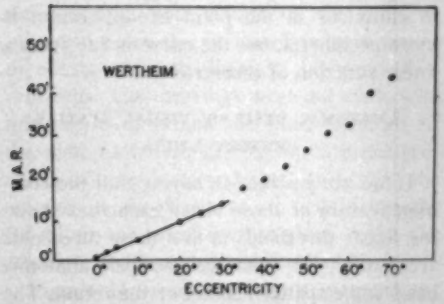

Fig. 5 (Weymouth). Minimal angle of resolution for temporal field as function of retinal eccentricity. Least square line fitted to values for 0 to 30 degrees inclusive. Data from Wertheim.

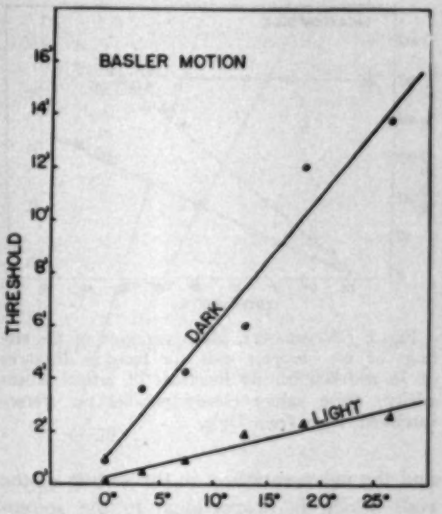

Fig. 6 (Weymouth). Threshold of motion measured in light and in dark as functions of retinal eccentricity. Data from Basler.

true of the vernier threshold (fig. 4), the perception of motion (fig. 6), and the values involved in the horopter such as the width of the haplopic zone (Panum areas, fig. 7),

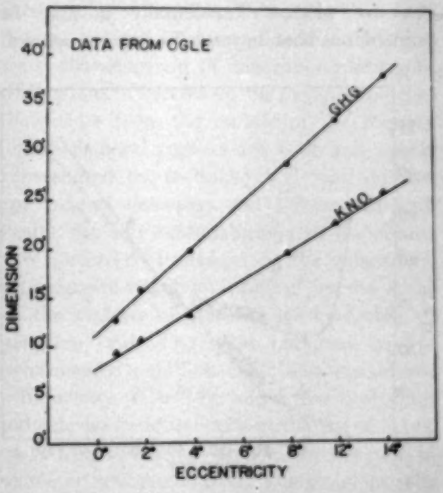

Fig. 7 (Weymouth). Diameter of Panum area for two subjects as functions of retinal eccentricity. Data from Ogle.

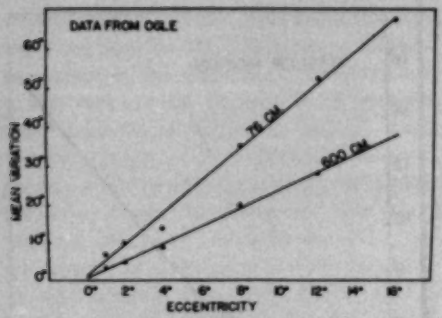

Fig. 8 (Weymouth). Mean variation of the settings of the horopter rods for fixation distances of 76 and 600 cm. as functions of retinal eccentricity; these values closely parallel the stereo-threshold. Data from Ogle.

and the mean variation in the setting of the rods which is proportional to the stereoscopic threshold (fig. 8).

All these thresholds may be stated in terms of visual angle either as distances on the retina or in comparison between the two retinas. It should again be noted that the linear type of spatial threshold is characteristic of the light-adapted eye only, as may be seen from Figure 9 in which the values of Fick for both the light- and dark-adapted eye are plotted. Incidentally it may be pointed out that beyond 30 degrees adaptation has little effect.

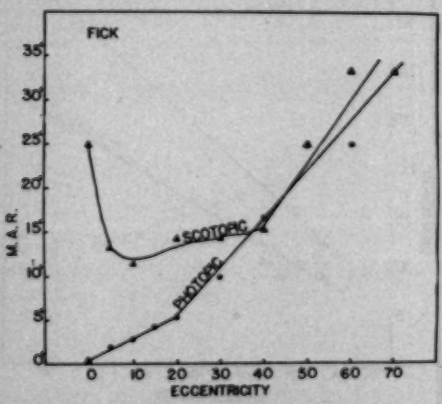

Fig. 9 (Weymouth). Minimal angle of resolution of the light and of the dark adapted eye as functions of retinal eccentricity. Data from Fick.

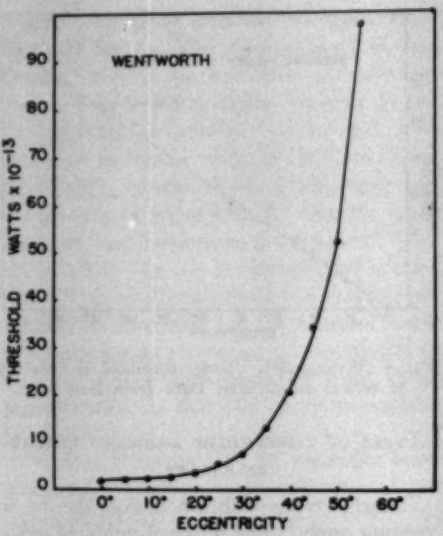

Fig. 10 (Weymouth). Light threshold (mean of four principal meridians) as a function of retinal eccentricity. Data from Wentworth.

Contrasted to these spatial thresholds linearly related to eccentricity is another group of thresholds such as that for light, the closely related pupillomotor threshold and the critical fusion frequency, which cannot be expressed in angular terms but may conveniently be considered intensity thresholds. These may be illustrated by the light thresholds of Wentworth (fig. 10). She found that progressively more energy was required to stimulate as the point of application is more peripheral, but the curve is far from a linear function of eccentricity.

Anatomic basis of visual spatial sensory units

If we are justified in saying that the common feature of those visual capacities showing linear thresholds is that these thresholds are spatial, we must seek as their anatomic basis some spatial feature of the retina. The spatial feature most often considered is cone density, for which are available the excellent data of Østerberg (1935) and Polyak

(1941). Thus, among others, Ludvigh and Polyak have plotted together visual acuity and cone density. Both decrease toward the periphery, if comparable scales are used; their relation will later be considered in more detail. Here it is sufficient to point out that physiologists accept some definition similar to the following, "the number of receptors connected by a single fiber to the brain . . . defines the extent of unitary field." (Piéron, 1952, p. 209.) If the optic nerve fiber or its cell of origin is taken as defining the retinal receptive unit, it is the density of the ganglion cell, not that of the cone, which should be related to the minimal angle of resolution or other thresholds which we are considering.

Ganglion-cell density

Unfortunately information on the density of the ganglion cells, although its logical importance is obvious, is far inferior to that for the rods or cones. The importance of ganglion cell density was recognized by Lashley in his study of vision in the rat (1932) and an analysis was attempted; but this aspect has usually been neglected. For man the work of Polyak (1941) is the best source of information, although no direct study was made and the data on ganglion-cell density are incidental. An analysis of this material was attempted. Drawings of retinal sections of the various regions are presented, and in these it was possible to make approximate counts of the ganglion cells and from the indicated magnifications to reduce these to distances between adjacent cells. The drawings were not made with this use in mind and the exact locations of the sections within the regions are not specified. Other uncertainties such as the amount of displacement of cells from the foveal to the parafoveal regions make the attempt merely an approximation.

Figure 11 is an attempt to present the data thus obtained. The calculated separation of the ganglion cells in minutes is plotted in the regions to which have been assigned the

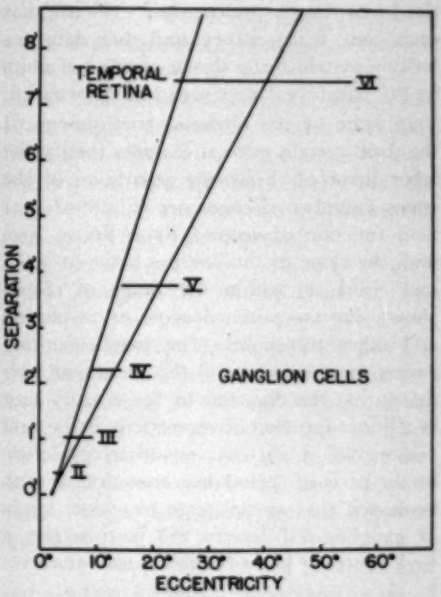

Fig. 11 (Weymouth). Ganglion cell separation as a function of retinal eccentricity; the two lines represent the two assumptions that the cell counts are characteristic of the inner margins or of the centers of the regions the width of which are represented by the horizontal lines.

eccentricity in degrees. Two lines have been drawn, one on the assumption that the slides were characteristic of the inner margin of the regions, a second on the assumption that they came from the middle of the regions. The peripheral regions are wide and poorly represented by sections; VII was omitted for lack of drawings and VI is used with hesitation. The reasonableness of the results was checked by transforming the separations into densities and multiplying by the areas of the regions to give the total number of ganglion cells. The value obtained, of approximately a million cells, was considered satisfactory. It will be noted that both lines indicate no ganglion cells in the fovea. This, of course, is true, an area 0.3 or 0.4 mm. in diameter is without cones. The ganglion cells for the sensory units of this area are found in the parafoveal region. For the center of

the fovea there is independent evidence that each cone is a sensory unit, but data are lacking by which the size or number of units in the parafoveal region can be determined.

In spite of the obvious shortcomings of the data, certain general features justify the labor involved. First, the separation of the cones (number of cones per minute of arc) as a function of eccentricity is linear. Second, the slope of the line lies between +0.2 and +0.3 or within the range of slopes shown for the minimal angle of resolution and other thresholds. The parallelism between the anatomic and the functional evidence that the diameter of the sensory unit is a linear function of eccentricity is the first reasonable, if sketchy, quantitative picture of the basis of spatial discrimination. It is to be hoped that an adequate histologic study of ganglion-cell density will in time give a firm anatomic basis for the spatial gradient.

Cone density and visual acuity

Let us return to the comparison of cone density and visual acuity. Polyak says, "Represented diagrammatically (fig. 99) the above figures of the size and separation of the cones form a curve similar to the visual-acuity curve of Wertheim and others, with a rapid fall close to the center and a slower decline toward the periphery of the field of view. The falling-off in the curve representing the cone gradient is, however, somewhat less steep than in the functional curve, suggesting a greater increase in the size of the functional cone units in the extra-foveal regions than indicated by the numerical distribution of the cones" (p. 436).

Although there is some confusion in the scales of the figure, the description is essentially correct. On another page he throws doubt on his own interpretation: "The belief of physiologists that the lesser peripheral visual acuity is due to several photoreceptors forming here a single physiological unit is still an assumption based at best only on computation. . . ." In the following analysis the logic of "computation" is rated rather higher.

For significant comparison, comparable scales are necessary. The visual acuity scale is based on an assumed "normal" angle of resolution of one minute of arc. The Snellen fractions are ratios representing the number of resolvable thresholds per minute; the Snellen decimal of 0.5 indicates that there is only half of a threshold per minute; that is, that the threshold is two minutes. The comparable scale for the cones would be the number of cones (better, the number of center-to-center cone separation) per minute. This is the reciprocal of the "separation" of cones as given by Polyak. Plotted on these scales as functions of eccentricity the comparison is shown in Figure 12A. The data of Fick have been used because the better known values of Wertheim are given only in the relative form, with foveal acuity set at 1.00. It will be seen that the foveal values differ little, but that as the periphery is

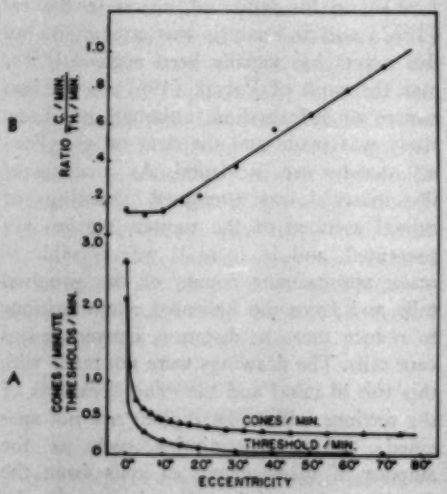

Fig. 12 (Weymouth). (A) Visual acuity (threshold/minute of arc) and linear cone separation (cones/minute of arc) as functions of retinal eccentricity. Visual acuity data from Fick; cone data from Polyak. (B) Ratio (cones per minute of arc/thresholds per minute of arc) as a function of retinal eccentricity.

invaded the "linear cone density," as the scale may be called, lies at a higher level than visual acuity. It is clear by "computation" that the sensory units, except at the fovea, must include more than one cone.

The ratio, cones per minute divided by thresholds per minute, should then give the number of cones in the diameter of a sensory unit; these ratios are given in Table 3 and are plotted in Figure 12B. It will be noted that in the fovea there is one threshold to one cone but that in the periphery the number of cones per sensory unit increases, as has often been repeated, as a linear function of the eccentricity to reach a value of over nine at 70 degrees, the limit of Fick's data.

The average of the ratios, weighted to take into account the areas involved, should be the ratio of the total number of cones divided by the total number of ganglion cells. The data, extrapolated to 80 degrees, give a weighted mean of 7.5; the number of cones by Østerberg's count is 6,550,000, the number of optic nerve fibers is 852,500 (Bruesch and Arey, quoted by Polyak); this gives a total ratio of 7.7.

Considering the wide range of published counts and the sources of error in comparing the acuity of one author with the cone density of a second, this strikingly confirms the previous analysis that the ganglion cells are the anatomic representatives of the sensory units and their regional distribution the basis of the linear relation of the threshold to eccentricity.

Earlier work

Has the linear relation of threshold to eccentricity been noted by earlier workers in this field? In Ogle's plots of the Panum areas on eccentricity (1950, p. 65, fig. 33) the lines connecting the points form linear regressions in some, in others there is a small concavity upward. He comments, "The relative proportion of Panum's areas to the peripheral angle is important. Figure 34 shows the curve which described the relationship. . . . The abscissa is the peripheral visual angle eccentricity and the ordinate is the ratio, expressed in percent, of the horizontal dimension of Panum's area to the visual angle. The curve decreases rapidly from the macula to a visual angle of four degrees, and then reaches a nearly constant value of about three percent for visual angles beyond five to six degrees."

Again on page 228 in Table 18 he gives as simple fractions the relative visual acuities as functions of eccentricity from Wertheim, Fick, and Ludvigh. In Figures 138 and 139 are plotted values derived from the table and mean deviations of the rod settings of the horopter for several workers for comparison with percentage magnification of images. Although a number of these approximate straight lines, this feature is not commented upon.

Ogle is impressed with the apparent constancy of the Panum areas as percentages of the eccentricity. If the threshold as a function of eccentricity were a straight line passing through the origin (this does not occur and would require an infinite foveal sensitivity) the threshold would be a constant percentage of the eccentricity. It is here claimed that these curves approximate a straight line, but with a finite and positive intercept; this would lead to a decreasing percentage, falling, at first, rapidly but changing more and more slowly in the periphery. The "constant" percentage relation noted by Ogle is therefore a consequence of the straight line relationship here discussed and is secondary and less useful mathematically. Although Ogle must have observed this linear relationship, he does not seem to have developed its consequences as is here done.

Interrelation of constants

In discussing the anatomic basis of spatial discrimination only the minimal angle of resolution has been considered, partly because of the more satisfactory data available. How do the other linear thresholds correlate

TABLE 2

CONSTANTS OF LINES FITTED TO SETS OF DATA FOR VARIOUS TYPES OF THRESHOLDS

No.	Fig.	Author	Date	Type of Threshold	No. Obs.	Ecc.	Slope	Intercept
1	—	Aubert & Förster	1857	(Data too scattered for fitting)				
2	5	Wertheim	1894	MAR temporal[1]	1	0°–30°	0.425	1.046[2]
3	9	Fick	1898	MAR horizontal	1	0°–20°	0.241	0.472
4	4	Bourdon	1902	Vernier	1	0°–20°	0.696	0.443
5	6	Basler	1906	Motion—light[3]	1	0°–26.5°	0.106	0.195
6	6	Basler	1908	Motion—dark[4]	1	0°–26.5°	0.502	0.988
7	3	Weymouth et al.	1928	MAR—ave. 4 meridians	1	0′–85′	0.406	0.614
8	1	Ludvigh	1941	MAR—horizontal (E.C.)	1	0°–10°	0.334	0.598
9	—	Ludvigh	1941	MAR—horizontal (ave.)	3	0°–10°	0.331	0.568
10	7	Ogle	1950	Panum area[5]	4	1°–12°	1.519	7.457
11	—	Ogle	1950	Panum radius[6]	4	1°–12°	0.759	3.729
12	7	Ogle	1950	Panum area (G.H.G.)	1	1°–12°	2.272	10.672
13	8	Ogle	1950	Mean variation—6 m.[7]	1	1°–12°	0.038	0.010
14	8	Ogle	1950	Mean variation—76 cm.	1	1°–16°	0.069	0.019
15	2	Original	—	MAR—students	20	0°–20°	1.504	1.469
16	—	Original	—	MAR—students (stand. dev.)	20	0°–20°	0.359	0.692
17	—	Original	—	MAR (St. error mean)	20	0°–20°	0.056	0.123
18	—	Original	—	MAR (Subject K.)	1	0°–20°	1.731	4.633
19	—	Original	—	MAR (Subject M.)	1	0°–20°	1.816	3.285

No. Obs. = number of observers.
Ecc. = range of eccentricity fitted—in some cases the original data covered a wider range.
Slope is in minutes of arc per degree.
Intercept is in minutes of arc.
[1] In all cases the MAR has been transformed from the original visual acuity.
[2] This value is relative and cannot be compared with other intercepts.
[3,4] Observations were made in the light and in the dark primarily to provide and to exclude comparison objects for the moving test object.
[5] The horizontal angular diameter of Panum areas as measured by the horopter method.
[6] The radius is half the horizontal diameter.
[7] Ogle has given the mean variation of the settings of the rods of the horopter apparatus instead of the standard deviation, the values do not differ much from the stereothresholds of the horopter.

with the retinal organization described above?

The slopes and intercepts of all the sets of data fitted are gathered together for comparson in Table 2. It will be noted that there is a tendency for both to increase or decrease together. The great heterogeneity of the data collected by many methods from many subjects over nearly 100 years precludes any close agreement. Three sets of data seem particularly aberrent.

Bourdon's data on the vernier threshold show a low intercept, corresponding to its great sensitivity, but a large slope; no cause for this is apparent, although it is stated that other workers have obtained a less rapid change in sensitivity with eccentricity.

In the second place, the student data on the minimal angle of resolution appear out of line (fig. 2 and table 1). Both slope and intercept are within the general range shown in Table 2, but the intercept is low for the slope. The high value of both is associated with the difficulty of the task (low illuminance and short exposure) but the discrepancy between them is perhaps related to the mass nature of the data since some individual records (K. and M., table 2) show a more usual relation of these constants. It is interesting that the variability of the student observations (standard deviation, table 2) shows a linear relation to eccentricity and the constants show the usual relations to each other.

The third case, that of the Panum areas, is of particular interest. The horizontal angular disparity of the haplopic or single vision zone of the horopter, which gives the diameter of the Panum areas, ranges from just short of uncrossed to just short of crossed diplopia, and is therefore equivalent approximately to two thresholds of the min-

TABLE 3

RATIO CONES PER MINUTE OF ARC AS A FUNCTION OF ECCENTRICITY THRESHOLDS PER MINUTE

Eccentricity (degrees)	Ratio (cones/thresholds)
0	1.000*
5	1.010
10	1.204
15	1.721
20	1.995
30	3.641
40	5.656
50	6.440
60	7.775
70	9.109

* This value of the ratio in the center of the fovea was not used in fitting the line of Fig. 12B.

imal angle of resolution type. It is clear that half of the horizontal diameter of the Panum "area" or the Panum "radius" should be used for comparison with thresholds.

The correlation of slope and intercept is +0.826. The data are 12 sets from Table 2, omitting the vernier threshold and some duplications, but including six sets of minimal angle of resolution, one standard deviation of minimal angle of resolution, two of motion, two of mean settings of horopter rods, and one Panum radius. This correlation is highly significant, it might be expected by chance from an uncorrelated population less than once in 1,000 times.

What is the origin of this correlation?

Although the slopes and intercepts vary widely, the relation between them tends to be consistent; they increase or decrease together, maintaining a constant ratio. Apparently the ratio is a function of the retinal organization, the absolute values, a function of the difficulty of the particular test. This assumption may be illustrated by an imaginary example.

Consider tests A and B depending upon the spatial organization of the retina; the threshold of A is a linear function of the eccentricity. Suppose that B proves easier than A so that at all eccentricities its threshold is only half that of A. B will then have a slope and an intercept both of which are half those of A.

An actual example is furnished by Basler's thresholds for motion. Tested in the dark without reference points with which the moving object might be compared, it proved about five times more difficult than in the light; the slope in the light is 21.2 percent of that in the dark, the intercept 19.7 percent, a discrepancy of only 1.5 percent from exact proportionality. Tests varying in difficulty or in the aspect of structure on which they depend will vary in this fashion, giving a highly correlated set of constants. If this analysis is correct it is plain that the arguments advanced for a structural basis of the minimal angle of resolution will apply with equal cogency to any other of the spatial thresholds considered in this paper.

USE OF CONSTANTS IN COMPARISONS

Having pointed out that a simple linear relation exists between a group of spatial visual thresholds and retinal eccentricity and that this relation has an anatomic basis, it may be well to illustrate the usefulness of this concept as an analytic tool. In Figure 13 are presented for comparison the plots of four types of visual thresholds and in Table 2, already cited, may be found the constants for the fitted straight lines. The minimal angles of resolution of Ludvigh and the vernier thresholds need no comment, the motion thresholds of Basler are in terms of extent of motion rather than rate, the values here plotted are those taken in the light and in the presence of reference points. Of the mean variation data Ogle says, "The setting of threads or rods of the horopter apparatus by the criterion of the apparent frontoparallel plane is essentially a task of stereoscopic depth discrimination" (p. 48). The conventional stereoscopic thresholds are larger than the values here given but undoubtedly parallel them. For near fixation points, for example, 30 or 40 cm., the mean variation is relatively large and as a function of eccentricity the curves are concave upward; for 76 cm. and 6.0 m. the thresh-

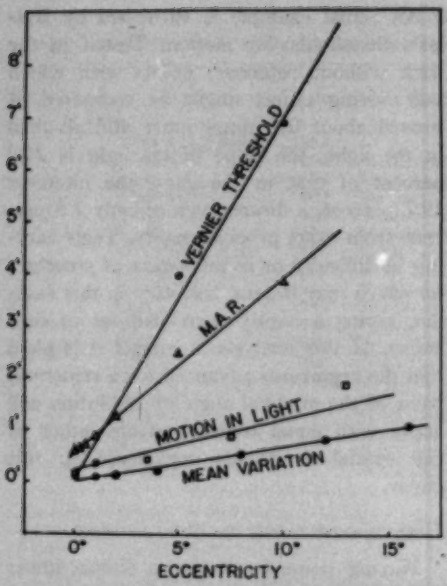

Fig. 13 (Weymouth). Comparison of vernier threshold, minimal angle of resolution, motion threshold, and mean variation of the settings of horopter rods; all are plotted as functions of retinal eccentricity. Data: vernier threshold, Bourdon; minimal angle of resolution, Ludvigh; motion threshold, Basler; mean variation, Ogle.

olds are much smaller and the curves are linear.

In spite of the heterogeneous nature of the data, certain important features are brought out by comparison of these curves. The vernier thresholds start lower than the minimal angle of resolution but increase more rapidly and by five degrees have crossed over to become the larger. Motion and the mean variation (or the stereothreshold) starting at about the same low foveal threshold as the vernier, on the other hand, increase the slowest of all (low slope) and so show in the periphery the lowest thresholds or the greatest sensitivity. The minimal angle of resolution is more often compared with movement and it is sometimes said that the preception of motion is better in the periphery than in the fovea. This is obviously not the case. The threshold of motion is smaller than the minimal angle of resolution in all parts of the retina. Owing to its lesser slope, however, its superiority markedly increases with eccentricity. This relative advantage over resolution in the periphery has apparently led to the idea that motion is more readily perceived in the periphery than in the fovea. The advantages of the linear representation of the visual thresholds, when it can be applied, are obvious in this example.

Summary

As is well known the retina of the light-adapted eye presents a field of graded sensitivity with its peak at the fovea. This has great theoretic and practical importance but accurate description and comparison have been hampered by the absence of a simple mathematical expression for the shape of the gradient. The minimal angle of resolution or threshold in minutes (the reciprocal of the visual acuity) plotted as a function of the eccentricity is a staight line rising from the lowest threshold in the fovea to high thresholds in the periphery. This is true, at least, for the central retina out to 20 or 30 degrees in all the data examined; beyond this it rises rather more rapidly.

The visual capacities may be divided into two groups:

First, spatial thresholds including the minimal angle of resolution, the vernier thresholds, the Panum fusional areas, the mean variation of the settings of the horopter rods (a function of the stereothreshold), the threshold of motion, and perhaps others. Second, intensity thresholds, including the light threshold, the critical fusional frequency, and probably others. The spatial thresholds of the photopic eye are linear functions of retinal eccentricity, the minimal angle of resolution of the dark-adapted eye and the intensity thresholds of both high and low levels of adaptation are nonlinear.

The linear relations of the spatial thresholds to eccentricity must have an anatomic basis in the spatial arrangement of the

retinal structures. If we accept the usual definition of a sensory unit that it includes a group of receptors communicating by a single nerve fiber with the brain, the optic nerve fibers or their cells of origin, the ganglion cells, rather than the cones, should be the anatomic basis of the spatial thresholds. From approximate densities of the ganglion cells in different regions of the retina it is found that the linear separation of the ganglion cells, and hence the diameter of the senory units, is a straight line function of the retinal eccentricity with a slope within the range of those of the spatial thresholds. If the ratio, cones per minute divided by thresholds per minute (derived from visual acuity), is compared for different regions of the retina it is found that peripheral to the fovea (in which the ratio is unity) the ratio is an increasing linear function of the eccentricity. These ratios, weighted by the areas of the retinal regions, give a mean ratio closely corresponding to the ratio of the total number of cones divided by the total number of optic nerve fibers. These relations are taken to prove that the anatomic basis of the linear arrangement of the group of spatial thresholds is a similar distribution of the ganglion cells, representing sensory units consisting of increasing numbers of cones with increasing eccentricity.

The usefulness of the linear relation to eccentricity of the spatial thresholds as a means of describing and comparing gradients is illustrated.

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OCULAR EFFECTS OF HIGH INTENSITY GAMMA RADIATION IN THE CAT*

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Histologic examination of a human eye that had received 13,000 r of gamma radiation revealed retinal vessels of arteriolar caliber that had undergone obliteration and fibrosis while vessels of similar caliber in the choroid appeared unaffected. Constriction of retinal vessels and retinal edema had also been noted clinically in this patient. These observations suggested the possibility that there might be a difference in the radiation sensitivity of retinal and choroidal blood vessels. In an effort to demonstrate such a difference, the eyes of three cats were exposed to heavy doses of gamma radiation. This paper summarizes the observations made in this preliminary investigation.

MATERIALS AND METHOD

Cats I and II were females, weight 2.72 and 3.14 kg., respectively. Cat III was a male, weight 2.82 kg. All three were maintained on the usual laboratory diet. Daily observation was carried out by one of us. The animals were killed by Nembutal injection. The eyes were fixed in formalin, paraffin sections were prepared, and the tissue studied with periodic acid-Schiff's stain, Weigert's stain, phosphotungstic acid-hematoxylin stain, and routine hematoxylin-eosin stains.

The cats were anesthetized or sedated (table 1) and immobilized in a plaster cast. The head was carefully shielded. Cat I was irradiated in a manner simulating the therapeutic dose schedule given the patient whose eye had shown obliteration of the retinal vessels. The right eye was given 19,900 r and the left eye 10,000 r. This was given in three courses of divided doses over a five-month period as shown in Table 1. After an additional five months of continued observation, the animal was killed and the eyes were studied as described.

Since Cat I showed only transient changes in the retinal vessels, Cat II was given a much more severe course of radiation, the right eye receiving doses of 36,000 r and 24,000 r in two sessions for a total of 60,000 r in nine days. The left eye was given a single dose of 24,000 r. The animal was observed for one month and killed. The eyes were prepared for histologic study. The data are summarized in Table 1.

Cat III received a single dose of 25,000 r to the right eye and 50,037 r to the left eye. This animal was killed after one week and the eyes prepared for histologic study. These data also appear in Table 1.

The radiation source was the General Electric Maximar instrument. Radiation factors were as follows: 250 k.v.p., no filter, half value layer 0.55 mm. Cu, distance 50 cm., field 2.0 by 2.5 cm. Accurate dosimetry was not attempted in this pilot study but tissue doses were considered to be within 10 percent of stated doses in air.

RESULTS

CAT I

During the first phase the right eye was subjected to 2,000 r and the left eye to 4,000 r given in divided doses over a four-week period (May 15, 1956, to June 16, 1956). Epilation became noticeable after only 800 r, and two months after the completion of treatment vitiligo became ap-

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TABLE 1

METHOD USED IN STUDY

	Dose Schedule (Doses)	(r)	Dates	Anesthesia	Total (r)
Cat I					
Phase 1					
R.E.	5	400	5/15/56 to 6/16/56	Ether	R.E. 2,000
L.E.	10	400	5/15/56 to 6/16/56		L.E. 4,000
Phase 2					
R.E.	3	200	8/21/56 to 8/27/56		R.E. 14,900
	2	250	8/23/56 and 8/24/56	Ether	
	1	1,200	8/28/56		
	4	2,400	8/31/56 to 9/7/56		
L.E.	1	1,200	8/27/56		L.E. 10,000
	2	2,400	8/31/56 and 9/4/56		
Phase 3					
R.E. (only)	2	2,500	10/26/56 and 10/30/56	Ether	R.E. 19,900 L.E. 10,000
Cat II					
R.E.	1	36,000	12/20/56	Thorazine	R.E. 60,000
	1	24,000	12/29/56	10 mg. per O.S.	
L.E.	1	24,000	12/29/56		L.E. 24,000
Cat III					
R.E.	1	25,000	3/21/57	Intraperitoneal	R.E. 25,000
L.E.	1	50,037	3/18/57	Nembutal	L.E. 50,037

parent. No other effects on the eyes were noted at this time (August 14, 1956). No increase in tearing or discharge and no evidence of conjunctivitis or iritis was noted. The lenses remained clear and the retinal vessels were normal to ophthalmoscopic examination.

During the second phase of treatment the right eye received 12,900 r, the left eye 6,000 r in a period of two weeks (August 21 to September 5, 1956). On September 6, 1956, attenuation of three of the four major arteries of the left eye was observed. There were no hemorrhages or exudates. The right eye was normal. On September 12, 1956, narrowing of three of the four arteries of the right fundus was evident. There was no other retinopathy. Considerable tearing and discharge developed in the right eye and prophylactic applications of Polysporin® ointment were begun. On September 18, 1956, mild yellowish discoloration and opacification of the superior portions of the fundi were noted. On September 25, 1956, anorexia developed and forced feedings were required for several days. Attenuation of retinal vessels gradually subsided during the next month. On October 22, 1956, early cataract was noted in the right eye. Epilation continued leaving thickened, white skin in the denuded areas.

During the third phase of treatment the right eye was given a further 5,000 r in two doses of 2,500 r on October 26 and October 29, 1956. The total radiation was 19,900 r to the right eye and 10,000 r to the left eye. Further irritation of the right eye occurred at this time with lid and conjunctival edema and discharge. There was no further change in the retinal vessels which had returned to normal before the final dose to the right eye was given and

remained normal until the experiment was terminated. In January, 1957, a radiation slough developed below the right eye and cataract appeared in the left eye. In both eyes the cataracts progressed only slightly. Observations were continued until March 27, 1957, when the animal was killed.

Pathologic examination of the eyes of Cat I revealed the following: *Right eye.* Lymphocytes, plasma cells, and polymorphonuclear leukocytes lightly infiltrate the conjunctival stroma. A few of the same cells are present in the superficial corneal stroma. The corneal epithelium is thinned to a layer three cells thick. Descemet's membrane and the corneal endothelium are not remarkable. The iris is normal. There is edema of the ciliary processes with fluid filled cysts of the pigmented epithelium (fig. 1). Lymphocytes and plasma cells lightly infiltrate the vascular layers of the ciliary body. The choroid and retina are not remarkable, and their vessels are normal. There is no evidence of degeneration of rods or cones. The lens is absent from the slides.

Fig. 1 (Winter, et al.). Edema of ciliary body and ciliary process in eye receiving 10,000 r over five-month period. Arrow indicates blisterlike collection of fluid between layers of ciliary epithelium. (Cat I, R.E., ×130, hematoxylin-eosin.)

DIAGNOSIS. (1) Mild, subacute conjunctivitis and keratitis; (2) mild, chronic iridocyclitis; (3) ciliary edema with epithelial cysts of ciliary processes.

Left eye. A few lymphocytes and plasma cells are present in the conjunctival stroma and there are a few polymorphonuclear leukocytes in the cornea. Descemet's membrane and the endothelium are not remarkable. The iris is normal. Edema cysts are present in the ciliary epithelium. The choroid is not remarkable. There are edema and microcystoid degeneration of the nerve fiber layer of the retina. Blisterlike formations are present on the inner limiting membrane of the retina and there are localized separations of the hyaloid. The retinal vessels are normal. There is absence of rods at the periphery on one side. Degeneration, flattening, and posterior migration of the lens epithelium are present and there are liquefaction and degeneration of the anterior and posterior cortical material.

Diagnosis. (1) Radiation cataract; (2) edema and cysts of the ciliary epithelium; (3) edema of retina.

CAT II

Cat II was given 10 mg. of Chlorpromazine® by mouth, the head was carefully shielded, and the animal was immobilized in a plaster cast. The animal received 36,000 r to the right eye on December 20, 1956, with no immediate systemic ill effect. On December 29, 1956, a further 24,000 r was delivered to each eye for a total of 60,000 r to the right eye and 24,000 r to the left eye.

By January 3, 1957, there was edema of the conjunctiva and lids of each eye with moderate discharge. There was hyperemia of the conjunctiva and dilatation of iris and ciliary vessels. There were heavy aqueous flare and cells. There was some haziness of media in the left eye but both fundi appeared normal. On January 5, 1957, anorexia

developed and no food was taken spontaneously after that date. There was heavy salivation and nasal discharge. The animal was lethargic. Equilibrium was poor. The eyelids were swollen and matted together.

On January 8, 1957, total detachment of the retina of the left eye was noted. Vessels of the upper half of the fundus, especially the arteries, were thinned and attenuated. The upper half of the fundus had a yellowish cast. There were no hemorrhages. The disc, retina, and vessels of the right eye were not remarkable. The animal was fed by gavage and additional fluids were administered by hypodermoclysis. Hydrocortisone and antibiotics were applied locally to the eyes in an attempt to prolong the period of observation.

On January 15, 1957, epilation was noticeable about each eye. The retinal arterioles were very narrowed or absent in the right fundus and only one vein could be identified. The retinal detachment persisted in the left eye and massive flame-shaped hemorrhages radiating from the disc suggested occlusion of one of the central retinal veins. The animal developed diarrhea and lost weight.

The animal was killed on January 18, 1957. At that time the right fundus revealed only one freely patent vessel, the inferior nasal vein. The superior nasal and inferior temporal veins were very narrow. The arteries could not be identified. There were no retinal hemorrhages but there appeared to be some retinal edema above. In the left eye the total retinal detachment was somewhat less elevated than before with extensive dark hemorrhages radiating from the disc.

Pathologic examination of the eyes of Cat II revealed the following:

Right eye. The corneal epithelium is reduced to a single layer in some areas. Otherwise, the stroma and endothelium are not remarkable. There is edema of the iris and ciliary body and a moderately heavy infiltration of lymphocytes and plasma cells is present in the trabecula, the iris, and ciliary body.

There are disorganization and fragmentation of the pigment epithelium of the ciliary body. The nonpigmented ciliary epithelium is disorganized and disoriented and there is marked nuclear and cytoplasmic pleomorphism. In many places there are blister-like collections of fluid separating the pigmented and nonpigmented ciliary epithelium (fig. 2). The choroid is not remarkable and the choroidal vessels including the tapetal capillary network are patent.

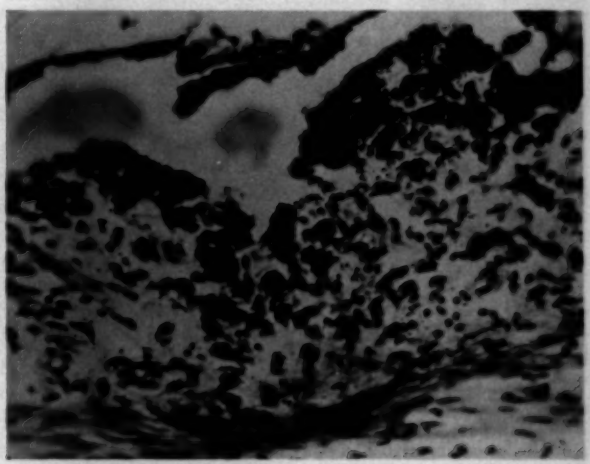

Fig. 2 (Winter, et al.). Disorganization of ciliary epithelium and formation of fluid-filled cystic space between layers of ciliary epithelium in eye receiving 60,000 r. (Cat II, R.E., ×450, hematoxylin-eosin.)

The rod and cone layer of the retina is completely obliterated and the outer nuclear layer has disappeared. A scattering of degenerating, swollen, or pyknotic nuclei and a few protoplasmic strands are all that remain of the outer retinal layers (fig. 3). There is no phagocytic or inflammatory infiltration and the inner nuclear and ganglion cell layers are not remarkable. There is edema of the nerve fiber layer with vacuolated spaces containing pink staining coagulum. The retinal vessels are patent. The optic nerve is not remarkable. The lens is absent from the slides.

DIAGNOSIS. (1) Chronic iridocylitis; (2) degeneration of ciliary epithelium; (3) degeneration of rod and cone cells of retina; (4) retinal edema.

Left eye. There is attenuation of the corneal epithelium. There is edema and diffuse hemorrhage in the iris and ciliary body and moderate lymphocytic and plasma cell infiltration of the iris, ciliary body, and choroid. Disorganization and degeneration of the pigmented and nonpigmented ciliary epithelium is especially pronounced. There are frequent blisterlike separations of the ciliary epithelium filled with granular coagulum. The choroidal vessels are engorged.

The retina is completely detached by massive subretinal hemorrhage. The retina itself is diffusely hemorrhagic. The retinal veins are dilated and engorged with blood. The rod and cone layer and outer nuclear layer have disappeared. There is degeneration of the cortical fibers of the lens at the equator and disorganization of the lens bow. The subcapsular epithelium is flattened, the cells are vacuolated, and there are swelling and pleomorphism of some of the nuclei. The optic nerve is not remarkable.

DIAGNOSIS. (1) Chronic uveitis; (2) degeneration of the ciliary epithelium; (3) degeneration of the rod and cone cells; (4) hemorrhagic retinal detachment; (5) cataract.

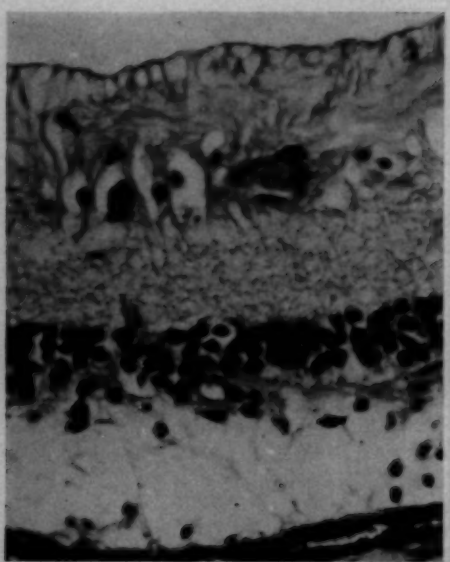

Fig. 3 (Winter, et al.). Degeneration of rod and cone layer of retina and edema of inner layers of retina in eye receiving 60,000 r. (Cat II, R.E., ×600, hematoxylin-eosin.)

CAT III

Cat III received a single dose of 50,037 r to the left eye on March 18, 1957. Within 48 hours there was marked swelling of the lids and edema of the conjunctiva. There was marked edema of the cornea and diffuse opacity of the lens. On March 21, 1957, the right eye was given 25,000 r in a single dose. Swelling of the lids and conjunctiva and opacification of the cornea and lens occurred before evidence of iritis or retinal vascular change could be noted. This animal was killed on March 26, 1957.

Pathologic examination of the eyes of Cat III revealed the following:

Right eye. There are edema and degeneration of the corneal epithelium which is absent in some areas. The anterior chamber contains a fibrinous exudate with moderate numbers of lymphocytes, plasma cells, macrophages, and red blood cells. The arteries and veins of the iris and ciliary body are tremendously dilated and engorged with blood, and there are marked edema and dif-

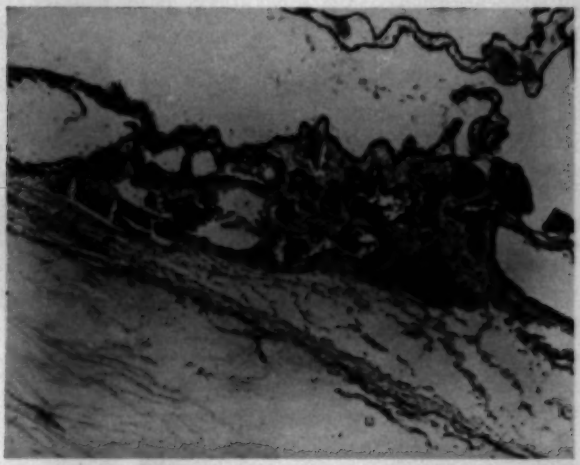

Fig. 4 (Winter, et al.). Marked edema and diffuse hemorrhage of ciliary body. Dilatation and engorgement of ciliary vessels five days after receiving 25,000 r. (Cat III, R.E., ×60, hematoxylin-eosin.)

fuse hemorrhage, especially in the ciliary processes (fig. 4).

There are fragmentation and degeneration of the pigment epithelial layer of the ciliary body. The nonpigmented epithelium is also undergoing disorganization with loss of polarity, cellular and nuclear pleomorphism, and variation in nuclear staining reaction. The choroid is not remarkable. The choroidal vessels are patent and contain red blood cells.

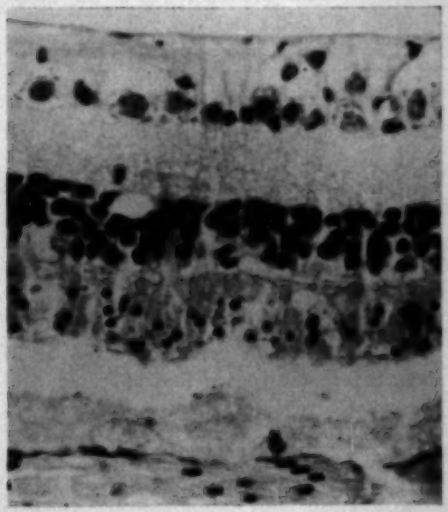

Fig. 5 (Winter, et al.). Degeneration of rod and cone layer and outer nuclear layer, clumping of chromatin in nuclei of ganglion cells, mild edema of nerve fiber layer, five days after 25,000 r. (Cat III, R.E., ×500, hematoxylin-eosin.)

The nasal half of the retina is well preserved. The cytoplasma of the rods is normal in appearance. There are numbers of pyknotic nuclei among the outer nuclear layer but in other respects the retinal architecture is not remarkable. In contrast, there is marked destruction of the rod and cone layer on the temporal side. The cytoplasmic processes of these cells are coagulated into a granular mass and the swollen and pyknotic nuclei are greatly reduced in number (figs. 5, 6, and 7). Edema and nuclear degeneration are also present in the outer nuclear layer. The ganglion cells are relatively little affected but some of their nuclei reveal clumping of chromatin. There is mild edema of the nerve fiber layer. There is degeneration of the subcortical lens fibers and edema of the lens epithelium at and behind the equator. The optic nerve is not remarkable.

DIAGNOSIS. (1) Acute keratitis and iridocyclitis; (2) vascular congestion, edema, and hemorrhage, iris and ciliary body; (3) degeneration of rod and cone cells; (4) retinal detachment; (5) cataract.

Fig. 6 (Winter, et al.). Degeneration of outer retinal layers eight days after exposure to 50,037 r. Arrow indicates patent capillaries of tapetal choriocapillary network. (Cat III, L.E., ×500, hematoxylin-eosin.)

Fig. 7 (Winter, et al.). Degeneration of outer retinal layers eight days after exposure to 50,037 r. Arrow points to patent capillary of outer retinal network. (Cat III, L.E., ×600, hematoxylin-eosin.)

Discussion

It was not possible to demonstrate under these experimental conditions any histologic difference in the radioresistance or radiosensitivity of retinal and choroidal blood vessels. While there were certain clinical differences in the response of retinal and uveal vessels, these were functional in nature and were concerned with such factors as dilatation or constriction of vessels and changes in permeability of the vessel wall. No structural differences were demonstrable pathologically.

Generally speaking, the response of the uveal vessels to radiation appears to be one of venous dilatation and increased permeability. This was evidenced clinically by engorgement of vessels of the conjunctiva, episclera, and iris and by the production of a plasmoid aqueous and small iris hemorrhages.

Histologically, it was also possible to demonstrate vascular engorgement, edema, and diffuse hemorrhage in the iris and ciliary body and fibrinous and hemorrhagic transudate in the anterior chamber. The approximate dose level to which the uveal vessels responded with dilatation was in the range of 800 r to 1,000 r and higher.

In contrast, the most striking response of the retinal vessels was constriction of the arterioles. This was demonstrable only upon observation of the retina with the ophthalmoscope and could not be demonstrated histologically with the techniques used. Venous change in the retina was also one of decrease in size of the lumen and followed the arterial constriction by some days. The threshold dose necessary to produce constriction of retinal arteries was in the order of 10,000 r. There was a latent period of seven to 10 days before the constriction appeared, and the vessels regained their normal size in 30 to 60 days.

Retinal edema accompanied the vascular change. Retinal hemorrhage was seen in only one eye and retinal detachment in two eyes. These changes occurred at a dose level of approximately 25,000 r. Histologically, edema of the inner layers of the retina

could be demonstrated together with vascular engorgement and retinal detachment. While functional alterations in blood vessel tone and permeability were easily seen, structural changes such as obliterative, thrombotic, or sclerotic alteration could not be found in either the retinal or uveal vessels. While it is generally understood that blood vessels are relatively sensitive to radiation damage, our failure to demonstrate distinct structural changes has been shared by others.[1-3]

All of the tissues of the eye with the exception of the sclera and the optic nerve showed radiation effects. Particularly striking was the degeneration of the photoreceptors already extensively studied by Cibis[4-6] and degeneration of the ciliary epithelium which has not as yet attracted full attention. Edema of the ciliary body and cysts of the ciliary epithelium were mentioned by Cibis, et al.,[5] and hypotension following irradiation has been noted by Biegel[7] and studied by Barber[8] and by Becker.[9]

In the two eyes exposed to 10,000 r and to 20,000 r in repeated divided doses over a period of months, alterations in the ciliary epithelium consisted of the formation of blisterlike separations of the pigmented and nonpigmented ciliary epithelium. These occurred over both the pars plana and the corona ciliaris and were especially prominent in the ciliary processes. In the more acute experiments, where doses of 25,000 r and greater were given, there were swelling and separation of cells of the pigment epithelium and disintegration of the pigmented layer. Granular coagulum collected between the two epithelial layers. Changes in the nonpigmented ciliary epithelium consisted of flattening of the cell layer, nuclear and cellular pleomorphism, and disorganization of the cellular pattern. The threshold dose for histologic change in the ciliary epithelium was in the range of 5,000 to 10,000 r while acute edema, disintegration, and disorganization occurred at 25,000 r and above. This histologic change took place within five days. Functional impairment of aqueous secretion occurs much more rapidly according to Becker[9] and to Biegel.[7]

Destruction of the rod and cone layer was striking in the animals receiving 20,000 r and more. In the eye receiving 10,000 r in divided doses there was no histologic change in the rods or the cones. Cibis[5] places the threshold for rod degeneration at 1,700 r to 2,000 r in such animals as the guinea pig, rabbit, dog, and monkey. It is interesting to note that when given in small divided doses the rod and cone layer of the cat withstood an amount of radiation at least five times that of the single threshold dose found by Cibis. At the dose levels employed in this study no difference in the susceptibility of rods to radiation damage was apparent.

Most of the other tissues of the eye also showed evidence of radiation damage. The corneal epithelium was edematous and thinned at doses of 25,000 r and over. Stromal edema occurred at 25,000 r and over. Degenerative changes found in the lens epithelium included flattening of anterior subcapsular cells, cellular pleomorphism, nuclear swelling and pyknosis, and chromatin extrusion at dose levels of 10,000 r and over. Cytoplasmic vacuolization and apparent edema of the epithelial layer occurred at 25,000 r. In addition to degeneration of the ciliary epithelium changes in the iris and ciliary body included edema and diffuse hemorrhage. Edema of the nerve fiber layer of the retina was demonstrable at doses of 25,000 r and over. Except for vascular dilatation and diffuse hemorrhage, the choroid seemed entirely resistant to damage at these dose levels.

Summary

A clinical and histologic study has been made to determine a possible difference in radiosensitivity of retinal and uveal vessels. Six eyes of three cats were exposed to gamma radiation varying from 10,000 to 60,000 r. There was a functional difference in response of uveal and retinal vessels, but no structural change in the vessels was demon-

strable histologically. Uveal vessels became dilated following single dose exposure of 800 to 1,000 r. This occurred within 24 hours and slowly disappeared in seven to 15 days. Constriction of retinal arteries followed by attenuation of veins was seen ophthalmoscopically three to five days after single dose exposures of 5,000 r to 10,000 r and passed off after 30 to 60 days. Histologically, both uveal and retinal tissue showed edema and diffuse hemorrhage.

As an incidental finding, marked degenerative change in the ciliary epithelium was noted on histologic preparations. This consisted of swelling and disintegration of the pigmented epithelium with nuclear and cellular pleomorphism. At doses in the range of 10,000 r, edema of the ciliary epithelium occurred with the formation of fluid-filled blisters between the pigmented and nonpigmented ciliary epithelium. Disorganization and destruction of the cells occurred at doses of 20,000 r and higher. Other tissue alterations were noted that have already been adequately described in the literature. These alterations included degeneration of the corneal epithelium, edema of the corneal stroma, degeneration of lens eipthelium, cortical cataract, edema and diffuse hemorrhage of iris and ciliary body, hemorrhagic and exudative retinal detachment, and marked degeneration of the rod and cone layer of the retina.

Stanford University Hospital (15).

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SECTION MEETINGS

of the

ASSOCIATION FOR RESEARCH IN OPHTHALMOLOGY, INC.

Auditor's Report

Officers
Directory of Members
Geographical List
Provisional Members

PUBLICATIONS COMMITTEE

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LORAND V. JOHNSON

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Eastern Section .....Bethesda, Maryland, January 17 and 18, 1957
Ludwig von Sallmann, M.D., *Chairman*

Midwestern Section .....St. Louis, Missouri, April 21, 1958
Bernard Becker, M.D., *Chairman*

Southern Section .....Miami Beach, Florida, November 12, 1957
Sherman B. Forbes, M.D., *Chairman*

Western Section .....San Francisco, California, November 1957
Levon Garron, M.D., *Chairman*

AUDITOR'S REPORT

Association for Research in Ophthalmology, Inc.

January 28, 1958

To the Members of the Board of Trustees
Association for Research in Ophthalmology, Inc.
Cleveland, Ohio

Dear Sirs:

I have examined the cash basis accounting records for the Association for Research in Ophthalmology, Incorporated, as of December 31, 1957, and the related transactions of receipts and disbursements for the year then ended. The examination was made in accordance with generally accepted auditing standards and included such tests of the accounting records and such other procedures as I considered necessary in the circumstances.

Despite the fact that no confirmation of dues in arrears was made directly with the members, in my opinion the accompanying statements present fairly the fund balances of the Association for Research in Ophthalmology, Incorporated, as of December 31, 1957, and the receipts and disbursements for the year then ended, in conformity with generally accepted cash basis accounting principles and applied on a basis consistent with that of the prior year.

Very truly yours,
MARTIN C. BLAKE.

The treasurer's report and the auditor's report was approved by the Auditing Committee consisting of Dr. Albert M. Potts and Dr. Levon K. Garron.

Balance on January 1, 1956			$ 7,218.14
Add: Receipt of Dues and Other Deposits			
Dues Collected		$9,371.00	
Interest from Government Bonds[1]		250.00	
Dividends from Union Electric Co.[2]		114.00	
Banquet Proceeds		695.00	
Supplement		2.00	
Canadian Exchange		1.06	10,433.06
Total of Cash Funds Accounted For			$17,651.20
Less: Disbursements			
Convention Expense:			
Dinners	$761.45		
Programs, notices, tickets, etc.	675.88		
Expense of Secretary	250.00	$1,687.33	
Publications—October, November		2,000.00	
Stationery, Supplies & Printing		212.09	
Postage		129.87	
Insurance Bond—$5.000.00 Sec'y-Treas.		12.50	
Bank Charges—Safety Deposit Box		6.29	
Telephone		133.32	
Secretarial Service		1,153.20	
Withholding Tax		88.20	
Rent for Office Space		540.00	
Meeting of Board of Trustees, June, Oct.		54.02	
Contribution—National Committee for Research in Ophthalmology & Blindness		500.00	
Friedenwald Memorial Lectureship		500.00	
Auditing Charges		115.00	
Copies of Supplements & Rosters		68.19	7,200.01
Cash Balance and In Bank on December 31, 1957			$10,451.19

[1] U. S. Treasury Bonds paying 2½% due 1957-72 having a carrying cost of $10,184.63.
[2] 75 Shares of Union Electric Co. @ 27⅜, initial value of gift from Julia Friedenwald Potts, $2,053.13.

Statement of Dues Collected for Year Ended 12-31-57

Dues collected for prior years		
1952—1 active member @ $5.00	$ 5.00	
1953—1 active member @ $5.00	5.00	
1954—1 active member @ $5.00	5.00	
1955—3 active members @ $5.00	15.00	
1956—2 active members @ $7.00	14.00	
1956—8 active members @ $10.00	80.00	
1956—2 educational members @ $3.00	6.00	$ 130.00
Dues collected for current year		
1957—750 active members @ $10.00	7,500.00	
1 active member @ $5.00	5.00	
28 sustaining members @ $25.00	700.00	
80 educational members @ $3.00	240.00	8,445.00
Dues collected for subsequent year		
1958—6 active members @ $10.00	60.00	
2 educational members @ $3.00	6.00	66.00
New members approved for 1958		
1958—17 active members @ $10.00	170.00	
1 sustaining member @ $25.00	25.00	
10 educational members @ $3.00	30.00	225.00
Provisional members for 1958		
1958—43 active members @ $10.00	430.00	
3 sustaining members @ $25.00	75.00	505.00
Total dues collected in year 1957		$9,371.00

Cash and Securities in Funds

	General Fund	Proctor Medal Fund	Friedenwald Memorial	Total
Cash 12-31-56	$ 7,218.14	—0—	—0—	$ 7,218.14
Cash 12-31-57	10,451.19	—0—	—0—	10,451.19
Increase	3,233.05			3,233.05
Securities		$10,184.63	$ 2,053.13	$12,237.76
Total Cash & Securities as of 12-31-57				$22,688.95

Summary of Membership by Years through December 31, 1957

Years Ended December 31	Total Members		
1957	979[1]	1944	283
1956	930[2]	1943	—[4]
1955	822[3]	1942	281
1954	815[3]	1941	279
1953	713[3]	1940	270
1952	672[3]	1939	268
1951	556[3]	1938	272
1950	509[3]	1937	249
1949	474	1936	240
1948	422	1935	245
1947	306	1934	230
1946	324	1933	219
1945	—[4]	1932	203
		1931	193
		1930	134

[1] Includes 28 new members for 1958, approved by the Board of Trustees on October 13, 1957. Does not include the 46 provisional members received between October 13, 1957, to December 18, 1957.

[2] Official membership whether dues paid or not.

[3] Includes only paid-up members.

[4] Statistical information not available.

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1958

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Schlaegel, Theodore F., Jr.
Taniguchi, Yoshiaki
Wilson, Fred M.

MUNCIE
Morris, Jean W.

NEW CASTLE
Burnett, Arthur B.

SOUTH BEND
Cassady, J. Vernal

WHITING
Apter, Julia T.

IOWA

CARROLL
Sullivan, John V.

CEDAR RAPIDS
Noe, Carl A.

CLINTON
Weih, Elmer P.

DES MOINES
Downing, Arthur H.

IOWA CITY
Alexander, Rose C.
Allen, Lee
Arnott, George P.
Blodi, Frederick C.
Braley, Alson E.
Burian, Hermann M.
Janes, Ralph G.
Leinfelder, P. J.
Richards, Richard D.
Schwartz, Bernard
Walker, Glenn L.
Whiteford, Robert D.
Wise, Arthur C.

MARSHALLTOWN
Wolfe, Dr. Otis D.
Wolfe, Russell M.

OSKALOOSA
Atkinson, George S.

SIOUX CITY
Reeder, James E., Jr.

KANSAS

ATCHISON
Bribach, E. J.

LAWRENCE
Fleming, David G.

KENTUCKY

FT. CAMPBELL
Portfolio, Almerindo G.

LOUISVILLE
Heitger, Joseph D.
Keeney, Arthur H.
Pfingst, Harry A.

LOUISIANA

FT. POLK
Christensen, Robert E.

NEW ORLEANS
Allen, James H.
Bahn, Charles A.
Bahn, Gustav C.
Barber, Aeleta N.
Boles, William M.
Byers, Jerome L.
Clark, William B.
Ellis, George S.
Haik, George M.
Holland, Malvern C.
Jimenez, Timoteo
Leckert, Edmund L., Jr.
MacDonald, Roderick, Jr.
Manning, John W., Jr.
Noble, Bertha R.
Rosenthal, J. William
Schoel, Robert E.

MAINE

BANGOR
Osler, Jay K.

LEWISTON
Tchao, Jou S.

PORTLAND
Maier, Paul

WATERVILLE
Dennis, Richard H.
Hill, Howard F.

MARYLAND

BALTIMORE
Abrahams, Irwin W.
Duke, James R.
MacLean, Angus L.
Maumenee, Alfred E.
Naquin, Howard
Patz, Arnall
Pogell, Burton M.
Rowland, Louise S.
Scholz, Roy O.
Stone, Howard H.
Woods, Alan C.

BETHESDA
Chalfant, W. Paxson, Jr.
Gunkel, Ralph D.
Hart, William M.
Livingston, Robert B.
O'Connor, George R.
Stone, Frederick L.
VonSallmann, Ludwig

CHEVY CHASE
O'Rourke, James F.

SILVER SPRINGS
Goodman, George

MASSACHUSETTS

BOSTON
Allen, Henry F.
Balazs, Endre A.
Beetham, William P.
Boruchoff, Saul A.
Casten, Virgil C.
Chandler, Paul A.
Chisholm, Julian E., Jr.
Cogan, David G.
Devi, Anima
Dunphy, Edwin B.
Grant, W. Morton
Gunderson, Trygve
Heath, Parker
Jakus, Marie A.
Liebman, Sumner D.
Murray, Edward S.
Regan, Charles D. J.
Regan, James J.
Schepens, Charles L.
Synder, John C.
Varga, Laszlo
Verhaeff, F. H.
Wachendorf, Fritz A. S.

BROOKLINE
Stone, William

CAMBRIDGE
Wald, George

LOWELL
Broggi, Richard J.

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Carpenter, Russell L.

NORWOOD
Ryan, William F.

SPRINGFIELD
Waugh, Richey L., Jr.

WORCESTER
Holzer, William F.
Myers, Roscoe W.
Whitney, Percy T.

MICHIGAN

ANN ARBOR
Alpern, Mathew
Falls, Harold F.
Fralick, F. Bruce
Henderson, John W.
Scharenberg, Konstantin
Smillie, John W.
Wolter, J. Reimer

BAY CITY
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DETROIT
Bialik, Michael H.
Burroughs, Roswell G.
Davies, Windsor S.
Guyton, Jack S.
Johnson, David S.
Kinsey, V. Everett
Lepard, C. W.
Lerner, Leonard H.
O'Rahilly, Ronan
Pugh, Howard C.
Reddy, D. Venkat
Ruedemann, A. D.
Ruedemann, A. D., Jr.
Schane, David
Scarney, Herman D.
Schimek, Robert A.
Sugar, H. Saul
Wachtl, Carl
Wehr, Maurice B.

JACKSON
McGarvey, William E.
Olesky, Stanley P.

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Petrohelos, Manousos A.

MINNESOTA

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Hilding, Anderson C.

MANKATO
Johnson, Hobart C.

MINNEAPOLIS
Fink, Walter H.
Halberg, Franz
Kantar, Bruce L.
McCannel, Malcolm A.
Schwartz, Virgil J.
Walsh, Francis M.
Wendland, John P.

ROCHESTER
Bair, Hugo L.
Benedict, William L.
Henderson, Dr. John W.
Hollenhorst, Robert W.
Martens, Theodore G.
Ogle, Kenneth N.
Rucker, Dr. C. Wilbur
Sabin, Frederic C.
Sheard, Charles
Stein, Harold A.
Steinmetz, Rodney D.
Winn, William E., Jr.

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Burch, Edward P.
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Tracht, Robert R.

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Lemoine, Albert N., Jr.
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Miller, Wade H.
Robinson, James T., Jr.
Shaad, Dorothy J.

MEXICO
Rouse, Daid M.

ST. LOUIS
Alvis, Edmund B.
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Becker, Bernard
Bryan, Dr. James H.
Cibis, Paul A.
Constant, Marguerite A.
Hartstein, Jack
James, William M.
Luedde, Philip S.
Mattis, Robert D.
Miles, Paul W.
Moses, Robert A.
Post, Lawrence T.
Post, Martin H., Jr.
Rosenbaum, Harry D.
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Schwartz, Frederick O.
Shahan, Philip T.
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Alliband, George T.
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Gifford, Harold
Judd, John H.
Lipp, Frank E.
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Stokes, William M.
Swab, Charles M.
Truhlsen, Stanley M.

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Magee, George R.

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Auten, Hanford L.
Sullivan, Paul B.

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Levy, Abram

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Meyer, George P.

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Goldsmith, Maximilian O.

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Nicholson, F. Peter

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Sherman, A. Russell
Sherman, Elbert S.

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D'Amico, Thomas V.
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Samuels, S. Lawrence

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Abrams, Henry

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Fonda, G. E.

SUMMIT
McAlpine, Paul T.

TEANECK
Berry, A. Erwin

TRENTON
Murto, Robert E.
Sacks-Wilner, Erwin
Sharbaugh, George B.
Wilner, Arthur S.

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Dillahunt, Jack A.
Schonberg, Albert L.

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BAY SHORE
Bussey, Frank R.

BAYSIDE
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BRONX
Burman, Daniel
Fleischner, Alois L.

BRONXVILLE
Koch, F. L. Philip

BROOKLYN
Bonadia, Calogero
Cholst, Mortimer
Evans, John N.
Fink, Austin I.
Grassi, Anthony J.
Levine, George
Mandelbaum, Joseph
Rosenberg, Abner S.
Rosenthal, Benjamin C.
Sands, Abraham M.

BUFFALO
Addington, Charles H.
Bennett, Arthur L.
Brennan, James W.
Buckheit, Rudolph G.
Fial, Edward A.
Fowler, James G.
Freeman, Sheldon B.
Howard, William M.
Jones, W. Yerby
LeWin, Thurber

Luhr, Alfred F.
Luhr, John P.
Naples, Ange S.
Olmstead, K. Elizabeth P.
Reitz, Herbert R.
Schopp, Robert C.
Smallen, Benjamin

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Hoffman, Parker M.

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Boland, William T.

FLUSHING
Kellerman, Leo D.

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Boyd, James L.

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Mintz, Maxwell A.
Ryan, Edward P.

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Presto, Ernest C.
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Morgana, Dante J.

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Chatzinoff, Albert B.

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Berens, Conrad
Bloomfield, Sylvan
Bonaccolto, Girolamo
Breakey, Arnold S.
Breinin, Goodwin M.
Burns, Robert P.
Byron, Herve M.
Campbell, Charles J.
Carroll, Frank D.
Carter, George Z.
Castroviejo, Ramon
Chambers, A. L., II
Chamlin, Max
Chang, Gilbert C. H.
Clark, Graham
Cole, Helen G.
Cole, John G.
Coles, Robert S.
Constantine, F. H.
Curtin, Brian J. H.
Day, Robert M.
DeVoe, Arthur G.
Dische, Zacharias
Dunnington, John H.
Epstein, Sidney S.
Esterman, Benjamin
Feinstein, Robert R.
Feldstein, Morris
Fernando, Antonio N.
Foote, Franklin M.
Friedman, Benjamin
Givner, Isadore
Gordon, Dan M.
Guy, Loren P.
Hartshorne, Isaac
Jacobson, Jerry H.
Kahan, Edmund
Kara, Gerald B.
Katzin, Herbert M.
Kaufman, Ira H.
Knapp, Arnold
Knapp, Arthur A.
Knapp, Phillip
Knighton, Willis S.
Koppel, Zoltan I.
Kornzweig, A. L.
Krohn, David L.
Kronenberg, Bernard
Krug, Joseph H.
Laval, Joseph
Linksz, Arthur
Locatcher-Khorazo, Deborah
Loewenfeld, Irene E.
Lowenstein, Otto
McLean, John M.
Macnie, John P.
Mamelok, Alfred E.
Marcus, Arthur A.
Merriam, George R., Jr.
Norton, Edward W. D.
Paton, R. Townley
Payne, Brittain F.
Perera, Charles A.
Posner, Adolph
Ralph, Fenn T.
Regan, Ellen F.
Reese, Algernon B.
de Roetth, Andrew, Jr.
Romaine, Hunter
Samuels, Bernard
Sauer, John J.
Schachat, Walter S.
Schachne, Lewis
Schlossman, Abraham
Shafer, Donald M.
Sitchevska, Olga
Smelser, George K.
Smith, Byron
Snyder, Stuart S.
Straatsma, Bradley R.
Sturman, Robert M.
Tabowitz, David
Theodore, Frederick H.
Troutman, Richard C.
Turtz, Arnold I.
Turtz, Charles A.
Tusak, Ervin A.
Webster, David H.
Weimar, Virginia Lee
Weintraub, Alfred
Wheeler, Maynard G.
Wise, George N.

NIAGARA FALLS
Goetzman, Arthur C.

OLEAN
Sheldon, Maurice G.

PLATTSBURG
Siegel, Edward
Smith, Ronald P.

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Baum, Gilbert

POUGHKEEPSIE
Cohen, Irving

ROCHESTER
Caccamise, William C.
Gipner, John F.
Lerman, Sidney
Snell, Albert C., Jr.
Sullivan, Charles T.

ROME
Reid, Frederick K.

RYE
Simonton, John T.

SCHENECTADY
Sykowski, Peter S.

SYRACUSE
Gillette, David F.
Katz, I. Herbert
McGraw, James L.
Marlow, Searle B.

UTICA
Valone, Richard J.

VALLEY COTTAGE
Cumming, Edith L. W.

WARSAW
Leachman, John W.

WATERTOWN
Atkinson, Walter S.

WEST ISLIP
Bergmann, Robert B.

NORTH CAROLINA

ASHEVILLE
Odom, Robert E.

CHARLOTTE
Ghent, Thomas D.

DURHAM
Anderson, Banks
Draheim, Jerry W.

GOLDSBORO
Bizzell, James W.

WINSTON-SALEM
Holt, Lawrence B.

NORTH DAKOTA

MINOT
Kohl, Darwin L.

OHIO

AKRON
Krichbaum, Franklin M.

ALLIANCE
King, George L.

CINCINNATI
Abrahamson, Ira A.
Abrahamson, Ira H., Jr.
Ascher, Charles K. W.
Brown, Albert L.
Freemond, Alan S.
Ginsberg, Joseph
Lyle, Donald J.
McGowan, William L.
Nelson, Robert C.
Reid, Horace W.
Sakler, Barnet R.
Schrimpf, Cyril Edward

CLEVELAND
Ballintine, Elmer J.
Bell, Richard P., Jr.
Bennett, James E.
Binder, Hertha
Binder, Rudolf F.
Brandwan, Samuel R.
Budd, Francis X.
Chamberlain, W. P., Jr.

Gans, Jerome A.
Gans, Morris E.
Guth, Sylvester K.
Hardesty, Hiram H.
Hare, James
Harvey, Elmer F.
Hertner, John E.
Johnson, Lorand V.
Kazdan, Philip
Kennedy, Roscoe J.
Keyes, John E. L.
Kirk, Robert C.
Kottler, Saul
Modrell, Robert W.
Nicholl, Russell J.
Nosik, William A.
Phillips, Josephine D.
Potts, Albert M.
Rosner, Robert S.
Schwarz, Gerald T.
Smith, Clarence G.
Thomas, Charles I.
Volk, David
Volk, Myron
Waitzman, Morton B.
Weekley, Robert D.
Wolpaw, Benjamin J.

COLUMBUS

Battle, Morris L.
Brecher, Gerhard A.
Ellerbrock, Vincent J.
Fry, Glann A.
Havener, William H.
Knox, George W.
Lipetz, Leo E.
Magnuson, Robert H.
Makley, Torrence A.
Prince, Jack H.
Stine, George T.
Suie, Ted
Taylor, Frank W.
Westheimer, Gerald

DAYTON

Insel, Herman H.
Schwarting, Bland H.
Teed, Roy William
Thomas, Edward R.

LAKEWOOD

Ellenberger, Carl

MASSILLON

Rummel, William D.

NEWBURY

Frohring, William O.

PARMA

Graham, John H.

SEVEN HILLS

Gilger, Anita P.

TOLEDO

Schmerl, Ernest
Skow, John D.
Steinberg, Bernhard
Vesey, Frank A.
Winger, Ira B.

UNIVERSITY HEIGHTS

Weinberg, Herman C.

WARREN

Chickering, Donald H.
Thomas, John H.

YOUNGSTOWN

Bloomberg, Louis
Evans, William H.
Ruth, Paul E.
Sovik, William E.

ZANESVILLE

Letson, Holton C.

OKLAHOMA

NORMAN

Wiley, George A.

TULSA

Lee, Otis S.
Ozment, Thomas L.

OREGON

COOS BAY

Flanagan, Roger M.

EUGENE

McCallum, George C.

PORTLAND

Allen, Aurelia J.
Browning, Charles W.
Butler, J. B. V.
Christensen, Leonard
Gruber, Louise
Harris, John E.
Heinrich, Daniel
Markley, Richard E.
Reeh, Merril J.
Swan, Kenneth C.
Taylor, E. Merle

ROSEBURG

Unruh, John W.

PENNSYLVANIA

ALLENTOWN

Beitel, Robert J., Jr.

ARDMORE

Vogel, Adolph W.

BEDMINSTER

Duane, Thomas D.

BELLEFONTE

Covey, John K.

BERWICK

Ross, Joseph V. M.

BRYN MAWR

McGavic, John S.

DANVILLE

Mahood, A. W.

ERIE

Alberstadt, Norbert F.

GETTYSBURG

Wickerham, Earl P., Jr.

GLENSHAW

Shaw, Harry E.

HARRISBURG

Martz, George E.

HAZELTON

Koven, Arthur L.

KITTENNING

Yockey, Robert H.

LANSDALE

Carmichael, Paul L.

MCKEES ROCKS

Terner, Irwin S.

NORRISTOWN

Tait, Edwin F.

PHILADELPHIA

Adler, Francis H.
Asbodian, Mila J.
Askovitz, Samuel I.
Blazar, Howard A.
Brav, Solomon S.
Cowan, Alfred
DeLong, Samuel L.
Ellis, Richard A.
Frayer, William C.
Gettes, Bernard C.
Green, Harry
Hallet, Joseph W.
Krewson, William E. III
Leopold, Irving H.
Lutman, Frank C.
Maynard, Robert E.
McDonald, Philip R.
Mullen, Carrol R.
Reese, Warren S.
Scheie, Harold G.
Sery, Theodore W.
Spaeth, Edmund B.
Spaeth, Philip G.
Tassman, Isaac S.
Trueman, Robert H.
Waldman, Joseph
Zentmayer, William

PITTSBURGH

Berk, Myles M.
Billings, Robert J.
Black, Henry H.
Bronk, Henry N.
Davies, Robert H.
Dunbar, John C.
Evans, Thomas M.
Everett, William G.
Goldberg, Sol
Grana, Phillip C.
Henry, Edgar S. H., Jr.
Hoffman, Franklin D.
Krieger, A. A.
Lehman, Robert N.
Linn, Jay G., Jr.
Linn, Jay G., Sr.
McCaslin, Murray F.
Novak, Joseph F.
Rohm, Robert F.
Shin, Ernest H.
Steinberg, Abraam
Thorpe, Harvey E.
Weisser, Charles W.
Wolken, Jerome J.

READING

Craig, Paul C.

SHARON

Bloomenthal, Sanford R.
Riddle, Ransford J.
Snyder, M. Wilson

SUNBURY

Ball, Donald N.

WARREN

Ball, William L.

RHODE ISLAND

PROVIDENCE
Stephens, H. Frederick

SOUTH CAROLINA

BEAUFORT
Sullivan, John J.

SOUTH DAKOTA

HURON
Kilpatrick, William R. J.

RAPID CITY
Palmerton, E. S.

YANKTON
Willcockson, Thomas H.

TENNESSEE

KNOXVILLE
Benedict, Walter H.

MEMPHIS
Krause, Arlington C.
Lewis, Phillip M.
Rychener, Ralph O.

NASHVILLE
Bounds, George W., Jr.
Zerfoss, Kate S.

TEXAS

AMARILLO
McKay, Edward D.

AUSTIN
Hilgartner, H. L.
Key, Samuel N., Jr.
Legett, Carey, Jr.

COLLEGE STATION
Feldman, Gerald L.

CORPUS CHRISTI
Gill, Earl K.
Stevenson, Roger

DALLAS
Browning, Carroll W.
Cary, Edward H.
Grafton, Edwin G.
Meisenbach, Albert E., Jr.
Melton, Carlton E., Jr.
Quinn, Lester H.
Thomas, Maxwell

EL PASO
Caylor, Robert N.
Schuster, Stephen A.

FT. HOOD
Chambers, Arthur L., II

FT. WORTH
Conner, William C.

GALVESTON
Gingrich, Wendell D.
Potter, William B.

HOUSTON
Daily, Louis, Jr.
Goar, Everett L.
Griffey, Edward W.
Leigh, Richard, Jr.
Vanzant, Thomas J.

McALLEN
Feink, Berton F.

PORT ARTHUR
Heare, Louis C.

RANDOLPH A.F.B.
Fixott, Richard S.
Taube, Jack I.

SAN ANTONIO
Matthews, John L.
Mims, James Luther, Jr.

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Davis, C. Truman
Veirs, Everett R.

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Mansur, Harl D., Jr.

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LOGAN
Lightfoot, Vernon F.

PROVO
Oaks, Lewis W.

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VERMONT

BURLINGTON
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NORFOLK
Crockett, Douglas H.

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ROANOKE
Stone, W. Conrad
Young, Charles A., Jr.

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McGuire, William P.

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Milam, Daniel F., Jr.

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Hill, Robert V.

SEATTLE
Jensen, Carl D. F.
Lyda, Wood
Peter, Philip A.
Young, Francis A.

SPOKANE
de Roetth, Andrew F. M.

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Ward, James F.

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Hogenson, Clifford D.
Stevens, Ralph W.

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Miller, Claude K.

WEST VIRGINIA

HUNTINGTON
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Polan, Charles M.

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Scott, Robert K.

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Ryan, Ralph W.

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LA CROSSE
Mansheim, Bernard J.

MADISON
Buesseler, John A.

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Haessler, F. Herbert
Schalmo, E. H.

FOREIGN COUNTRIES

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Pang, Herbert G.

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Margarida, Carlos Jose

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Duggan, John W.

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Marshall, M. R.

MANITOBA
Elvin, Norman L.

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Rosen, David A.
Dyson, Charles

NIAGARA FALLS
Eager, R. F.

TORONTO
Elliot, Alfred J.
Levitt, Herbert
McCulloch, Clement
MacDonald, Roy K.
Ormsby, Hugh L.
Shusterman, Morris

MONTREAL
Alexander, Benjamin
Boxer, Louis
Brault, Jules
Cloutier, Roland
Locke, John C.
Viger, R. J.

VANCOUVER
Irving, John A.
Mallek, Howard
Patterson, John W.
Ragan, John D.

VICTORIA
Wiggins, Robert L.

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BAHIA
de Andrade, Cesario

MIÑAS GERAIS
Dasilva, Antonio I.

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Alvaro, Moacyr E.

CANAL ZONE

ANCON
Mahaffy, Steve R.

COSTA RICA

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de la Cruz, Edgar

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Ferrer, Horatio

PERU

LIMA
Barrere, Luciano E.
Raffo, Julio C.

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CARACAS
Grom, Edward

PHILLIPINE ISLANDS

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De Ocampo, Geminiano

QUEZON CITY
Quilala, Francisco V.

BELGIUM

GHENT
François, Jules

ENGLAND

LONDON
Duke-Elder, Lady Phyllis
Duke-Elder, Sir Stewart

FINLAND

HELSINKI
Koistinen, Aune Marjatta

ISRAEL

JERUSALEM
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ITALY

CAGLIARI
Grignolo, Antonio

JAPAN

HONGO
Hagiwara, Hogara

SOUTH AFRICA

JOHANNESBURG
Taylor, Israel B.

SWITZERLAND

GENEVA
Blum, John

TURKEY

ISTANBUL
Sezer, F. Necolet